*The Pathology of*

# DIABETES MELLITUS

BY

**Shields Warren, M.D., ScD.**

Departments of Pathology of the New England Deaconess Hospital and the Harvard Medical School, Boston, Massachusetts; Director of the Division of Biology and Medicine, U. S. Atomic Energy Commission

AND

**Philip M. LeCompte, M.D.**

Departments of Pathology of the Faulkner Hospital and the Harvard Medical School, Boston, Massachusetts

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# PREFACE

THE abnormalities of function of vital importance to the diabetic patient are intimately related to changes in cell and tissue structure. As new techniques in histochemistry and histology have been developed our ability to relate and understand these structural and functional alterations has been materially increased. Our knowledge of diabetes has been far from static since 1938, and many new factors have been developed. This third edition is larger than previous editions in spite of elimination of much old material as a result of the continuing advances that have been made in the field. These advances are particularly important in the field of experimental diabetes, in the understanding of the changes encountered in infancy and childhood, and in special pathology, particularly that of the kidney, the eye and endocrine glands.

No one man's knowledge can hope to be complete in this field, and the authors are deeply indebted to many for help in varying degree. Without the continuous stimulation of Dr. Elliott P. Joslin the many obstacles incident to a task such as the preparation of this book would have been even more obvious. Drs. Alexander Marble, Howard F. Root, Priscilla White, Francis D. W. Lukens and Stanley Robbins have been most helpful and have read one or more chapters. Valuable material or helpful suggestions have been received from Drs. David Cogan, Arnold Lazarow, H. Berning, Jonas S. Friedenwald, G. Lyman Duff, G. Gomori, E. T. Bell, E. L. Stubbs, Frank Bloom, J. B. Hazard and G. Kenneth Mallory. Furthermore, without the day by day help of those working with us in the technical and secretarial fields, the preparation of the volume would have been extremely difficult. We are indebted to Miss Nancy Buck, Mrs. Diane Crocker and Mrs. Mildred Reilly for histologic technical assistance, to Miss Jane Hennelly for compilation of tables, to Mrs. Kathryn Haley for photomicrographs and proofreading, and to Mrs. Jean LeCompte for assistance in the preparation of the manuscript and proofreading.

SHIELDS WARREN
PHILIP M. LECOMPTE

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# THE PATHOLOGY OF DIABETES MELLITUS

## Chapter 1

## DIABETES MELLITUS AND THE PANCREAS

The name insulin given the pancreatic hormone has by suggestion so thoroughly convinced us of the truth of the insular hypothesis that it is well to examine briefly the evidence upon which it rests at the present time and to see whether we are justified in accepting it in its entirety. The importance of the truth of this theory with regard to the etiology, treatment and prognosis of diabetes mellitus is obvious. Although Cawley[1] in 1788 reported a case of diabetes that came to autopsy and showed marked pancreatic damage ("the pancreas was full of calculi which were firmly impacted in its substance . . . the right extremity of the pancreas was hard and appeared to be scirrhous") the relation of the pancreas to diabetes was not recognized for another century. A few scattered observations[2,3] suggested the importance of this gland in diabetes but failed to attract any general attention. Only with the discovery of von Mering and Minkowski[4] that removal of the pancreas would cause diabetes in experimental animals was the relationship definitely established. Even so, the occurrence of apparently normal glands in many diabetics and the absence of diabetes in individuals much of whose gland had been destroyed by necrosis or by malignant disease were thorns in the flesh of the pathologists for twenty years and even now there are twinges from the wound. Moreover, Minkowski's experiments showed that the presence of only one-eighth of the pancreas was sufficient to avert the development of diabetes in animals so that the pancreatic lesions found in human cases of diabetes hardly seemed sufficiently severe.

Laguesse[5] hazarded the opinion that the islands of the pancreas were organs of internal secretion, basing this on anatomical grounds, especially the relation of the epithelium of the islands to the rich vascular network that surrounds it. The first extensive pathological study was that of Hansemann[6] in 1894 who collected 72 cases from the literature and presented 54 new cases. However, this group was studied chiefly from the standpoint of gross pathology. Hansemann's tables are given below. The granular atrophy of Hansemann is identical with Opie's interacinar fibrosis. It is of more than passing interest that the islands of Langerhans are not mentioned in Hansemann's tables. Indeed, Hansemann was the last as well as the chief champion of the acinar hypothesis, assuming that

TABLE 1

| *Cases from the Literature* | | *Cases from the Berlin Pathological Institute* | |
|---|---|---|---|
| Simple atrophy | 18 | Granular atrophy | 36 |
| Chronic interstitial inflammation | 18 | Fibrous induration with hypertrophy | 3 |
| Pancreatic calculi | 15 | Complicated case | 1 |
| Necrosis (? of post-mortem change) | 6 | Normal pancreas | 8 |
| Carcinoma | 5 | Condition not noted | 6 |
| Lipomatosis | 3 | | |
| Fatty degeneration | 3 | | |
| Abscess | 2 | | |
| Cyst | 1 | | |
| Hyperemia | 1 | | |
| Total | 72 | Total | 54 |

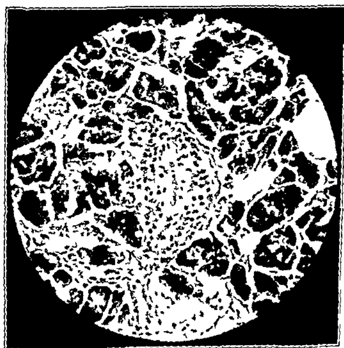


FIG. 1.—Moderate hyalinization of island of Langerhans. Male aged sixty-six years. Duration of diabetes fourteen years.

the acini were the seat of control of carbohydrate metabolism and consistently opposing the insular hypothesis.

The transplantation of the remaining portions of partially resected pancreases by Hédon[7] with the failure of diabetes to develop as long as the transplants remained in healthy condition served to show that an internal secretion and not the external was concerned in carbohydrate metabolism. The observation of Opie,[8, 9] later confirmed by Ssobolew,[10] localized in the islands the pathological changes associated with diabetes and explained, at least in part, the previous difficulties in correlation of the clinical and pathological findings. In cases of diabetes mellitus the islands were frequently diseased with or without damage of the acinar tissue, while in cases of extensive pancreatic lesions with normal carbohydrate metabolism, such as some cases of acute pancreatic necrosis or carcinoma, the islands were found to be intact. The abnormalities in the islands were either qualitative, as sclerosis or hyalinization, or else quantitative. Ssobolew described complete absence of islands in 4 out of 13 cases of diabetes. This finding is most remarkable. Probably if he had had the advantage of the specific staining methods for the island cells discovered by Lane,[11] Bensley,[12] and Gomori,[13, 14] he would have found at least some island tissue present, perhaps in the form of islet cells scattered through the acinar tissue, as sometimes occurs.

Another earlier report[15] of "absence" of islands is uncertain, as the author failed to find islands in two nondiabetic cases as well as the one of diabetes. Likewise, Binger and Keith[16] reported a case of apparent complete absence of islet tissue in a nondiabetic. No instance of complete absence of insular tissue checked by specific staining methods has been reported.

In 1904 Sauerbeck,[17] analyzing the available evidence, championed the insular hypothesis and it has remained the favorite up to the present. Opie[18] presents a table (Table 2), based on cases compiled by Sauerbeck and himself.

Heiberg[19] and Weichselbaum[20, 21] each claimed pancreatic changes to be present in every case of diabetes and to be limited to the islands in a sufficient number of cases to prove the insular hypothesis. Cecil,[22] in a series of 90 cases, came to the same conclusion and later[23] called attention to the frequent occurrence of evidence of regeneration or hypertrophy in the islands of Langerhans in diabetes (34 out of 100 cases). Similar changes suggesting hypertrophy and regeneration were found in cases of chronic pancreatitis without diabetes. Yotuyanagi[24] has described new formation of islands from centro-acinar cells as well as regenerative hypertrophy in cases of severe pancreatic damage.

The presence of regenerative processes in the islands was also stressed by Weichselbaum[25]—both cellular proliferation of the island epithelium and the formation of new islands from the epithelium of the ducts. With all this evidence favoring the insular hypothesis there was nevertheless an undercurrent of discouragement, of dissatisfaction with the available means

TABLE 2.—PANCREATIC LESIONS SHOWN BY CASES COMPILED BY SAUERBECK AND OPIE

| | |
|---|---|
| Interacinar pancreatitis | 125 |
| Interlobular pancreatitis | 13 |
| Lipomatosis | 18 |
| Calculi | 9 |
| Cyst | 1 |
| Carcinoma | 5 |
| Focal necrosis | 2 |
| Atrophy | 65 |
| Lesions of islands with normal parenchyma | |
| Hyaline degeneration | 6 |
| Sclerosis | 2 |
| Adenoma-like hypertrophy | 3 |
| No lesion of pancreas | |
| Pancreas normal | 34 |
| Number of islands diminished | 5 |
| *Total* | 288 |

of recognizing characteristic lesions in diabetes. This feeling was well expressed by Allen[26] nearly forty years ago and his statement even today holds true: "Every anatomical hypothesis of diabetes still requires to be assisted by assuming the existence of a certain proportion of functional cases without known anatomical basis."

With the differentiation by Lane[11] of $\alpha$ and $\beta$ cells in the islands of Langerhans, and with the beautiful studies of Bensley[12] on the pancreas of the guinea pig, it was natural to attempt to correlate cytological with clinical findings in the hope that there might be found some single universal diabetic lesion. Homans[27] found disappearance of the secretory granules in the island cells of cats from which three-fourths of the pancreas had been removed. In those animals which became diabetic he noted "hydropic degeneration" of the island cells, and hence regarded them as the site of the carbohydrate-regulating function. This "hydropic degeneration," previously noted by Weichselbaum[25] in diabetic patients and which Allen[6,28] had produced experimentally in dogs, Homans[29] found to affect chiefly the $\beta$ cells.

This gave new hope for the existence of a single characteristic universal diabetic lesion. Further study has unfortunately blasted this hope. Hydropic change of the $\beta$ cells may well mean diabetes when it occurs, but by no means all diabetic pancreases show this lesion.

In recent years considerable interest has been shown in the quantitative relationships between the two major types of islet cells. As will be noted in Chapter 3, differential counts of the ratio of $\beta$ to $\alpha$ cells by various more or less specific staining methods have led to conflicting results, some observers (Ferner,[30] Hess[31]) claiming to find a striking and diagnostic reduction in the ratio, others (Gomori,[13,32] Terbruggen[33]) finding in many cases ratios either entirely within normal limits or showing only slight deviations.

The duct ligation experiments of Schulze[34] Ukai[35] and others[36,37] showed that extensive (though now known to be incomplete) atrophy of the acinar tissue was unaccompanied by disturbance of carbohydrate metabolism

Insulin[38] was the climax of this line of experimentation In spite of the important evidence adduced by the production of insulin from the principal islets of fish[39,40] and from pancreatic tissue in which the acini had practically disappeared secondary to duct ligation, the production of insulin in the entire absence of acini was not yet proved

It remained for Wilder's[41] striking case of hyperinsulinism due to carcinoma of the islands of Langerhans to clinch the truth of the insular hypothesis This was further reinforced by the carcinoma of Thalhimer and Murphy[42] the adenoma of McClenahan and Norris[43] and by the results of the operative removal of the carcinoma of island-cell type reported by Howland[44]

Of some interest in this connection are cases of naturally occurring diabetes in animals Spontaneous diabetes in dogs has been reported by Bloom and Handelsman[45] and Bloom has observed spontaneous diabetes associated with hyaline islands in the cat[46] More recently Landé[47] has described the case of a four-year-old cat which developed thirst and excessive urination and at autopsy showed small islets with some hydropic and myxomatous changes In the case of Pollock and Bauman[48] a six year-old Irish setter exhibited polydipsia polyuria and weight loss and at autopsy showed an enlarged pancreas (65 gm) in which the islets were

we found a peculiar massive coagulative necrosis of the pancreas and a liver almost replaced by fat According to Bloom (personal communication) a fatty liver is characteristically found in both canine and feline diabetes Certainly as noted by Pollock and Bauman[48] more attention should be given to these interesting cases

Schlotthauer and Millar[49] report 9 cases in dogs and cats and note that the disease may be due to pancreatitis or may be idiopathic They illustrate hyaline islets in one cat and state that most diabetic dogs are females and most diabetic cats are males

According to Dr I I Stubbs[50] the incidence of diabetes in domestic animals as judged from various series reported in the literature is probably highest in dogs but even here is quite low most authors giving figures of less than $\frac{1}{10}$ of 1 per cent and many as low as 1 in 10 000 He also cites the interesting statement of Runnells[51] that diabetes is believed to be the cause of death in feeder lambs when overfed on carbohydrates such as corn sugar molasses If this conclusion be true the important implications in connection with the experimental work of Dohan and Lukens[52] and the role of diet are obvious (Chapters 25 and 26)

Perhaps the most convincing and dramatic evidence in favor of the insular hypothesis is the remarkable specific damaging effect of alloxan on the $\beta$ cells of the islets and the intimate correlation between this damage and the development of diabetes in animals so treated (*see* Chapter 25). Other bits of evidence include the correlation of lowered insulin content of the pancreas with injury to the islets,[53] the lesions of the islets in pituitary diabetes,[54] the degranulation of the $\beta$ cells in experimental hyperglycemia[52,55] and in human diabetes,[56] the changes of the mass of islet tissue in response to treatment with glucose or with insulin,[57] and the fact that diabetics excrete less insulin in the urine than do nondiabetics.[58]

## THE ACINO INSULAR HYPOTHESIS

That the malfunction of the pancreas as a whole is responsible for diabetes is the essence of the acino insular hypothesis. Lombroso[59] presented evidence for this theory. In the hands of some other proponents, such as Reitmann,[60] who believed that normally islands and acini both controlled carbohydrate metabolism, yet either alone could perform this function, and Karakascheff,[61] who believed the islands were only of use to replace worn-out acini and were not separate organs, this theory was decidedly weak. While Laguesse[62] valiantly upheld the possibility of reciprocal transitions between acini and islands, he nevertheless believed the islands to be primarily concerned with carbohydrate metabolism. This hypothesis is a logical corollary of the transition theory of interrelation of islands and acini which will be discussed in Chapter 2.

A monograph of Seyfarth[63] sums up the evidence and perhaps marks the height of the prestige of this theory. Gomori[64] has summarized the points of view of the two opposing schools of thought in relation to the interdependence of acinar and islet tissue, and has presented his own reasons for doubting the supposed conversion of acinar cells into islet cells and vice versa.

## THE ACINAR HYPOTHESIS

Of historical interest alone is the belief of Hansemann[6] that the acinar cells are the seat of regulations of carbohydrate metabolism. He laid great stress on granular atrophy, essentially interacinar fibrosis, as the most important lesion in diabetes.

## DEFECTS IN THE PANCREATIC THEORY

In recent years there has been increasing realization that the whole picture of human diabetes cannot be interpreted solely in terms of the pancreas, and much has been made of the fact that many cases of the dis-

diabetes due to adrenal tumors have been permanently cured by removal of the tumors[66] and finally that total pancreatectomy in man results in a relatively mild form of diabetes[67 68] On the other hand it must be recognized that all known forms of *permanent* experimental diabetes are associated with lesions of the islets and that probably the great majority of human cases would show lesions if studied with proper histologic methods and quantitative techniques It is interesting that such a prominent authority as Wilder[69] in a recent important paper offers a spirited defense of the unitary pancreatic view of human diabetes Perhaps the soundest point of view is to regard the majority of cases as fundamentally pancreatic (though modified by various factors) and to recognize a small group of definitely extra pancreatic origin based perhaps primarily on dysfunction of the liver or adrenals If one adopts the point of view of Himsworth[70] and regards diabetes mellitus as a *syndrome* rather than a disease entity much of the difficulty of thinking of it in anatomical terms is obviated (*see* Chapter 26)

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## Chapter 2

# THE NORMAL PANCREAS

THE relation of the pancreas—particularly its islets—to carbohydrate metabolism is so marked as to necessitate a thorough understanding of the normal histology of the gland in any consideration of the anatomical pathology of diabetes mellitus.

Few organs of the body are less constant in size and architecture than the pancreas. The descriptions given in most textbooks of anatomy cover the essential features of the gland but give altogether too great an impression of its constancy in morphology. For example, the weight at autopsy in individuals showing no evidence of pancreatic disorder ranges from 60 to 160 grams, averaging about 95 grams. One of the best descriptions of the pancreas is that of Opie[1] and this has been freely drawn upon in the present discussion.

In the loop of the duodenum lies the head of the pancreas. From the upper and anterior aspect of the head a constricted portion, the neck, bends slightly upward and forward and then stretches to the left, forming the body of the gland. Behind the neck pass the superior mesenteric vessels. The body tapers slightly toward the spleen and this thinner portion of the gland is called the tail.

The development of the pancreas takes place from three primitive outgrowths from the mid gut, one of which is on the dorsal surface between the liver and the stomach and the other two a right and a left ventral, which subsequently fuse and rise on either side of the hepatic duct and then grow to meet the dorsal part. With the alteration in position of the stomach and duodenum the pancreas is changed in position so that the dorsal portion lies transversely, with its left extremity near the spleen, and the ventral portion is carried with the torsion of the duodenum to the right and backward. Thus with further growth the part of the pancreas which was originally ventral comes in contact with the larger dorsal part and unites with it, as a rule, during the second half of the second month of embryonic life.

In studies on 21 human embryos, Pearce[2] found the islands to appear first in the 54 mm. embryo as oval eosinophilic cell masses continuous with the acini. During the third month of development a few completely separated islands were found, while most remained connected with the acini by solid stalks of cells. Each island was provided with four to seven afferent and efferent capillaries. Development is not infrequently arrested in congenital syphilitic pancreatitis and islands are found still communicating with acini and ducts. In light of the studies of Bensley and others the

islands probably have a common origin with the acini from the duct cell rather than arising from the acinar cells However, the acino-insular relationship is still disputed[3] Neubert[4] in a detailed study of the development of the pancreas held that the islets arise from the secreting ends of the adenomeres as well as from the ducts This extensively quoted work is considered by Kraus[5] and others to offer a reasonable explanation of the acino insular relationship and at least to imply the possibility of new formation of islets from acinar tissue in the adult Hughes[6] from a study of alloxan diabetes holds that formation of islet cells from acini occurs in the rat (*see page 27 for further discussion*) *Gomori*[3] *points out that* much of the evidence for transformation of acinar cells to islet cells is based on insufficient evidence or inadequate technique

Ferner[7] has worked extensively with the Gros-Schultze method of silver impregnation and has studied the islets at all stages of development He holds that the silver positive cells include the $\alpha$ cells of the islets and in addition cells scattered singly and in groups among the acini in the connective tissue and in the ducts In the embryo he describes buds of these silver cells arising from both ducts and acini and refers to them as being inselpotente *i e* the precursors of islets In early developing islets he describes the appearance of a central nucleus of $\beta$ cells which do not take the silver surrounded by the unripe silver positive cells The silver cells predominate in early life in fact are exceedingly numerous at birth then decrease relative to the $\beta$ cells through about the fourth year after which time the relationship becomes more or less stabilized According to this point of view the silver cells including the $\alpha$ cells are resting non functional elements from which $\beta$ cells may be formed under the proper stimulation Further discussion of Ferner's ideas is given later in this chapter and in Chapter 3

Hard[8] working with rat embryos and using the neutral gentian method of Bensley and Gomori's modification of the Mallory Heidenhain stain found that the $\beta$ cell was the only islet cell type to differentiate during embryonic life and that the specific granules could be distinguished at about the nineteenth day *i e* fairly late in fetal life The $\alpha$ cell (rather surprisingly in view of Ferner's statements noted above) was not demonstrable at all during fetal life being recognized first on the second postnatal day

The pancreas is traversed from left to right by the duct of Wirsung which bends downward and backward in the neck and after traversing the posterior surface of the head reaches the wall of the duodenum where it unites with the common bile duct The accessory pancreatic duct that of Santorini arises in the upper portion of the head and terminates in a small papilla in the mucous membrane of the second portion of the duodenum about 2 cm above the papilla of Vater

There are numerous variations in the distribution of the ducts and their anastomoses In 100 pancreases Opie[1] found the ducts in anastomosis in

90 He gives a number of excellent diagrams of the relationships of the two ducts

On the surface of the pancreas irregular areas are demarcated by loose connective tissue and fat The amount of this fat varies greatly with the state of nutrition of the individual and is one of the chief factors in the variability of weight of the gland The gland can be divided by dissection of the tissue into clear cut lobules usually about 1 cm in diameter These primary lobules are subdivided into smaller secondary lobules usually several millimeters in diameter by poorly-defined septa With the low power of the microscope these secondary lobules may be still further divided to tertiary lobules

Aberrant pancreatic tissue may occur anywhere in the upper gastro intestinal tract Faust and Mudgett[9] review the literature and report a case in which a mass of pancreatic tissue presented as a polyp of the stomach

The blood supply of the body and the tail of the pancreas is from branches of the splenic artery, whereas the head receives its supply from the superior pancreatico-duodenal artery a branch of the hepatic and from the inferior pancreatico-duodenal artery a branch of the superior mesenteric The bulk of the venous blood from the pancreas passes into the splenic vein and a smaller amount into branches of the superior mesenteric vein Anastomoses are frequent both arterial and venous An excellent review is given by Kirk[10]

The gland is innervated by fibers from the henal plexus which in turn is formed by branches from the left celiac ganglion the celiac plexus and right vagus nerve Pacinian corpuscles are not rare Sympathetic ganglion cells may be found though rarely in all parts of the pancreas

## THE HISTOLOGY OF THE PANCREAS

The following description of the pancreatic cells is based on the study of human autopsy material fixed from twenty minutes to three hours post mortem No pancreas showing gross evidence of pathological change was included In addition the information disclosed by the far more careful researches of Bensley[11] Laguesse[12] Ukai[13] and many others has been liberally drawn upon

Supposedly normal pancreatic tissue was fixed routinely in Zenker's fluid sublimate-bichromate and formalin Frequently Bouin's fluid Champy's fluid Zenker formol aceto-osmic-bichromate osmic acid absolute alcohol and Regaud's method were also resorted to

The following stains were used

Phloxine-methylene blue
Phosphotungstic acid hematoxylin
Mallory's aniline blue
Goodpasture's eosin methylene blue

Masson's iron hematoxylin vert lumière
Iron hematoxylin Van Gieson
Ukai's stain
Bensley's neutral gentian
Gomori's chrome-alum hematoxylin and phloxine
Gomori's new aldehyde-fuchsin method
Gros-Schultze silver method (slightly modified from Ferner)

On certain favorable cases various lipoid stains were employed as were *Best's and Langhans' methods for glycogen. When the tissue was fresh* and properly fixed the aniline fuchsin methyl green or picric acid method or the iron hematoxylin method for mitochondria was employed as were Bensley's safranin stain and a very useful modification of Mallory's aniline blue stain devised by Bensley.[14]

Recently we have had considerable success with Gomori's chrome-alum hematoxylin and aldehyde-fuchsin methods perhaps the most generally useful stains for the islets yet devised. On well fixed material his modification of the Mallory Heidenhain azocarmine method is also of value. Also we have made extensive trials of Ferner's[7] method of applying the Gros Schultze silver stain to the pancreas and have had good results with a slight modification of it. This may yet prove to be the most useful method for estimating in autopsy material the relative numbers of $\alpha$ and $\beta$ cells. Unfortunately it only works well on frozen or celloidin sections. Our ef*forts to develop a workable silver method for paraffin sections have* met with limited success.

Details of the more useful methods are given in Appendix A. A critical discussion is given by Gomori.[8]

**Duct Cells** The simplest system in the pancreas is that of the ducts with which may be included the centro-acinar cells. Moreover from the primitive duct epithelium the external and the internal secretory elements of the gland are derived. As would be expected from their origin from the duodenal epithelium the ducts at their proximal ends are lined by epithelium closely resembling that of the duodenum—tall columnar cells with distally placed elongate nuclei. Granules varying in size which give the characteristic staining reactions of mucin occasionally occur in the proximal portion. True goblet cells occur with varying frequency. Near the duo-
apil
arity
ving

At times even the neoplasms derived from the two are practically indis
relatively thick

scattered

The mucous secretion of the cells disappears and the epithelium gradually changes from columnar to cuboidal as the ducts become finer. There is a tendency for the duct epithelium to flatten slightly in the intercalated ducts and frequently the centro-acinar cells flatten very abruptly. The centro-acinar cells are similar in appearance to the cells of the finer ducts and probably represent the termini of the ducts. They tend to insinuate themselves about the apices of the acinar cells.

Jacoby[15] demonstrated alkaline phosphatase in the duct epithelium by histochemical methods and postulated that the ductules were the source of this enzyme, the other three enzymes of the pancreatic juice being produced by the acini. Wang, Grossman and Ivy[16] confirmed the histochemical observations and showed further that pancreozymin stimulates the output of amylase from the pancreas but not that of alkaline phosphatase.

Both duct cells and centro-acinar cells have a clear acidophilic cytoplasm free from secretory granules and chromidial substance. In favorable cases scattered fine rod-shaped mitochondria are visible in both duct cells and centro-acinar cells. Nassonov[17] demonstrated a simple Golgi apparatus in these cells. It is much less complicated than in the acinar and island cells.

The nucleus is usually oval, smaller than that of the acinar cell, and relatively rich in chromatin. The nucleolus, so prominent in the acinar cell, *is not obvious. Extremely rarely mitosis is seen, even in adults.*

Bensley[11] has demonstrated in the guinea pig, through vital staining with pyronin, a system of fine anastomosing tubules surrounding the larger ducts and communicating with the ducts on the one hand and the islands of Langerhans on the other. Moreover, scattered in these tubules are single cells identical with those of the islands. Otani[8] found in human cases direct connection between a moderate proportion of the islands and the interlobular or intralobular ducts.

**Acinar Cells.**—That portion of the pancreas with which we are best acquainted is the external secretory system. The obvious importance of the pancreatic ferments and the striking appearance of the acinar cells early focussed attention on the cytology of the latter. The easy demonstration of the zymogen granules made attempts at correlation between changes in the intracellular structure and glandular secretion peculiarly enticing.

With any of the ordinary stains the cytoplasm of the acinar cells is clearly divided into two zones. The inner zone is markedly vacuolated and with appropriate staining these vacuoles are seen to contain, or rather be, the *zymogen granules. The outer portion of the cell, usually containing the* nucleus, stains strongly with basic dyes and is faintly striated. Thus when stained with Goodpasture's methylene blue after chromesublimate or Zenker-formol fixation the outer portion of the cell shows a diffuse bluish clouding (chromidial substance) with faint clear striations. These striations are known to be due in animals to the presence of mitochondria. While we have had great difficulty demonstrating mitochondria in human pancreatic

tissue in several cases they have had this same distribution. Acetic or osmic acid in the fixing fluid causes the chromidial substance to be precipitated in filamentous form (Bensley[11]). However, Saguchi[9] believes these filaments are in reality preformed and simply made visible by the acid.

In the human cases in which mitochondria are found they appear as fairly long, slightly wavy rods of moderately irregular thickness arranged more or less parallel to the long axis of the cell and only very rarely occurring in the inner or vacuolated zone. We have not been able to detect any definite relation between mitochondria and zymogen granules.

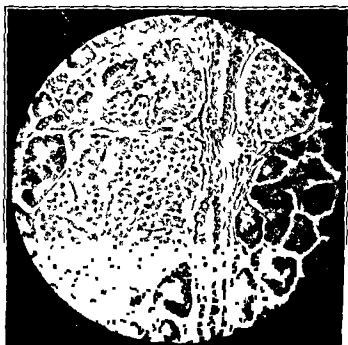


Fig. 2. Normal pancreas showing acini, island and duct. Male aged forty-six years. No diabetes. Phosphotungstic acid hematoxylin stain. × 170.

With stains such as phosphotungstic acid hematoxylin following formalin fixation or as Bensley's neutral gentian, the zymogen granules *are the most prominent elements of the cell*. These granules vary in size but are fairly coarse. Their origin is still in dispute, being considered as mitochondrial derivatives, products of the Golgi apparatus or of the chromidial substance. Even the nucleus has been thought their source.

Occasionally there are scattered foci of cells ranging from a portion of an acinus to several adjacent acini in size which stand out markedly acidophilic with eosin methylene blue staining. These cells have a clear homogeneous cytoplasm and apparently do not contain either zymogen

granules or chromidial substance These are more likely foci of exhausted cells rather than foci of necrosis They throw some light on the supposed transition of acinar to island tissue as in casual examination of a section they strongly resemble islands However, these cells never contain the specific granules of the island cells and their arrangement is in no way characteristic of islands They are presumably identical with the groups of eosinophilic cells described by Hamperl[20] in various organs especially salivary glands parathyroid and thyroid and called by him onkocytes Curiously, Hamperl does not mention the pancreas in his latest paper [0] on "onkocytes" although previously[21] he placed some emphasis upon it as a common site of these cells At any rate it would seem that little is to be gained by labeling these cells since by so doing no light is cast upon their nature or function

**Island Cells** —Distributed irregularly in the pancreatic parenchyma are the groups of small polygonal cells with homogeneous cytoplasm first described by Langerhans[22] These cell groups vary greatly in size but most are between 75 and 175 micra in diameter The variation in number is also great Bensley[11] has counted as many as 56 000 in the pancreas of the guinea pig and found an average of 22 3 per c mm in adult guinea pigs Clark[23] found from 250 000 to 1 750 000 in the pancreases of non diabetic adult humans Owing to variation in distribution even in the same pancreas estimates of the number of islands present are of but little value They are said to be more frequent in the tail than in the remainder of the organ

A quantitative study of the pancreatic islands was made by Ogilvie[24] He used an ingenious method for calculating the area and weight of the islets by projecting microscopic fields on a sheet of paper tracing the outlines of the islets weighing both the intact sheet and the cut-out islets calculating the volume by assuming the average islet to be a sphere and finally applying a formula to calculate the weight of islet tissue in relation to the weight of the pancreas Forty five fields were examined from each of 39 pancreases from females and 41 from males ranging from newborn to sixty four years of age The average weight of the pancreas increased from 2 6 grams at birth to 66 grams for those over twenty-one years of age while the average total weight of the islands increased from 0 12 grams at birth to 1 07 grams for those over twenty-one years of age However the insular weight varies through a wider range than does the total pancreatic weight For the first two years the rates of increase of islands and of total pancreas are parallel and greater than the rate of body growth From four to twelve years of age the rate of increase of islet tissue is about one-half that of the total pancreas and the body as a whole but at adolescence the rates are again equal

After the third year Ogilvie found the number of islands to remain fairly constant the weight of the average island increasing from 0 369γ at birth to 1 468γ in adult life The weight varies over a wide range even in adults

from 0.478γ to 2.738γ. When the islands are relatively few, or when they are more numerous than average, they tend also to be small. Larger

117,226 in a year-old

Many pancreases in adult life contain about 1,000,000 islands, but the figures vary nearly as widely as those just given. Heiberg[25] estimated that the islands made up approximately 3 per cent of the pancreatic tissue. Bargmann[26] gave counts in 5 adults varying from 208,369 to 1,760,000.

The most careful quantitative study of the islands of Langerhans is that of Tejning,[27] who followed the effect of diet on the volume of the islet organ in rats. He used serial celloidin sections 30 micra thick of the carefully weighed formalin fixed pancreas, projecting and tracing the islets in every tenth section, and applying the mathematical methods of Wicksell for calculating the volume of ellipsoidal solids. He found a definite correlation between the total volume of the islets and the numerical and volumetric distribution of islets of different magnitude. He also found a positive correlation between the size of the islet organ and the pituitary, and a sex difference in that the female rats tended to have a larger islet organ both on normal and special diets. His results on the size of the islet organ as influenced by various diets are of great interest and are summarized in the accompanying table.

TABLE 3 (FROM TEJNING[27]). VOLUME OF ISLET TISSUE OF RATS EXPRESSED IN CU MM PER 100 GM BODY WEIGHT

| | *High Carbohydrate* | *High Protein* | *High Fat* | *Natural Diet* |
|---|---|---|---|---|
| Males | 0.711 ± 0.022 | 0.594 ± 0.028 | 0.472 ± 0.021 | 0.673 ± 0.026 |
| Females | 0.889 ± 0.028 | 0.684 ± 0.030 | 0.504 ± 0.023 | 0.742 ± 0.020 |

Tejning[27] has criticized (and apparently with some validity) the work of all previous investigators in the quantitative field, including Bensley[11] and Ogilvie,[2] as involving technical errors, inadequately controlled unusual material or unjustified mathematical assumptions. For the details of his arguments and methods reference should be made to his paper. He has undoubtedly made a valuable contribution in his critical survey of the field and his emphasis on the extreme care that must be exercised in order to obtain valid results in this difficult line of investigation.

Methods for estimating the volume of the islets in small animals have also been described by Mount[29] and by Haist *et al.*[29] both involving pressing the pancreas between glass plates (the latter authors also using neutral red injection), then projecting with a micro-pro[illegible] tracing the islets and calculating the volume and weight of the [illegible] ation to the whole pancreas. These metho[illegible] ly subje[illegible] ng s criticism that all the islets, especially [illegible] ma[illegible] fied. The work of Haist *et al.* is discu[illegible] Chapt[illegible]

Susman[30] applied a somewhat similar method to paraffin sections of human pancreas (*see* Chapter 3)

Thompson *et al*,[31] from a study of the frequency distribution of the volume of the islands in man monkey, and dog hypothesized that no new islands are formed after prenatal life

Until the work of Bensley[11] the distinguishing characteristics of the island cells were chiefly negative the most important being the absence of zymogen granules Specific granules had been demonstrated in the island cells by Laguesse[17] in 1896 and the presence of two different types of cells having been detected by Schulze[33] and Diamare[34] the path for further study was opened by Lane[35] who devised methods for distinguishing the two types of granules from one another and also from the zymogen granules

Two types of island cells are commonly recognized the $\alpha$ cells and the $\beta$ * Unstained the granules of the two different types of cells cannot be distinguished from one another The granules of the $\alpha$ cells are stained violet by safranin acid violet following Lane s chrome-sublimate fixation and the $\beta$ granules are stained red With the same fixation and acid fuchsin after the neutral gentian stain the $\alpha$ cells are red and the $\beta$ cells violet With the modification of Mallory s aniline blue which we have found very useful, the $\alpha$ granules are stained red and the $\beta$ granules are stained bluish purple (*See* Plate I ) This same result is obtained by Ukai s stain With Gomori s chrome-alum hematoxylin stain the $\beta$ cells are gray-blue with

various

e island

cells were not preserved or were not stained the protoplasm was finely alveolar and that as post-mortem degeneration progressed a coarser alveolar structure appeared This has an important bearing on the question of hydropic degeneration

In addition to the presence of specific granules the types of island cells can be distinguished by nuclear characteristics Both types of island cells lack the prominent nucleus of the acinar cell The nuclei of the $\alpha$ cells are poor in chromatin and are usually oval in outline The nuclei of the $\beta$ cells are usually spherical though sometimes slightly oval and contain relatively large granules of chromatin In addition to these two types of cells cells are found without granules whose nuclei closely resemble those of the $\alpha$ cells The $\beta$ cells form the greater bulk of the island Gomori[3] one of the leading students of the pancreas states that in normal human islands about 60 to 90 per cent of all cells are $\beta$ cells 2 to 8 per cent D cells and the rest $\alpha$ cells

* Lane[35] who gave the first definitive description of these cells called the cells which he thought were fixed by alcohol ' A cells and those fixed by his aqueous chrome sublimate solution $\beta$ cells Bensley[11] writing shortly afterward called them A and B There seems to be a tendency in the recent European literature to use the latter nomenclature However since it is customary in this country to speak of alpha and beta cells we have chosen to use the Greek letters

In the guinea pig Bensley[11] found no definite arrangement of the $\alpha$ and $\beta$ cells in the islands. They also occur without definite arrangement in island of the human pancreas, although the $\alpha$ cells appear a little more frequently at the periphery of the islands.

Tschassownikow[36] suggested that the $\alpha$ cells were intermediate between the acinar cells and the $\beta$ cells. However, Bensley[11] has shown that they occur in large numbers in the interstitial islands which are developed directly from the ducts; they are present in the interior of the island as well as at its periphery; they are present in the acini of newborn guinea pig without $\beta$ cells, and they disappear from the acini within the first week of life without any increase in the number of $\beta$ cells. Bensley concludes that *the two types are independent and that each develops from an undifferentiated duct cell.* Bloom[37] found a type of cell which one of us (S. W.) described in the first edition of this book (page 36) to be so constantly present that he described it as a third type of island cell, *the D cell*, which is about as frequent as the $\alpha$ cell. The D cell granules stain light blue after formaldehyde chrome-sublimate fixation and the Mallory azan stain.[38]

These observations were confirmed by Gomori[3] who stated that he found definite transition forms between the D and $\alpha$ cells. In three papers[3, 39, 40] this author has given an admirable summary of the application of specific staining methods to the pancreas of numerous animals as well as man. The D cells are not demonstrable with the neutral stains of Lane, Bensley and Bowie, nor are they shown by Gomori's chrome-alum hematoxylin phloxine stain, which is, however, excellent for distinguishing the two main cell types. *They are shown by his new aldehyde-fuchsin trichrome method.*

In addition to the granules the island cells contain mitochondria which appear as delicate filaments and fine granules. They are scattered throughout *the cell in marked contrast to their concentration at the base* of the acinar cell. Among the granules of the island cells are lipoid droplets and also clear canals which may be identified by appropriate staining as Golgi *apparatus*.

O'Leary[41] has followed *in vivo* the formation of vacuoles within the island cells and the migration of these vacuoles toward the capillary end of the cell where they gradually decrease in size. It is possible that these may be secretory vacuoles. It may be that the appearance of "hydropic degeneration" is due to the formation of large numbers of these vacuoles, and in view of Toreson's work cited in the next chapter the possibility of their containing glycogen must be considered.

Saguchi[19] has described in the frog's pancreas five types of island cells which have transitional forms between certain of them. In human tissue we have been unable to parallel Saguchi's types.

Throughout 41 representative mammalian species Thomas[4] has found $\alpha$, $\beta$ and D cells. The C cell occurs only in the guinea pig. In this range of species the size and *organization of the islands differ widely.* Differential

PLATE I



NORMAL PANCREAS

$\alpha$ cells contain orange-red granules. $\beta$ cells light blue granules. D cells [illegible] granules. The duct epithelium stains a very light blue. The acinar cells stain [illegible] purple. By a slight modification the zymogen granules can be shown [illegible] [illegible]

[illegible]

[illegible] to be due to the presence of fat. In [illegible] with Figure [illegible] shows that the amount of fat demonstrated there is not excessive. No. 31[illegible]. Female aged fifty seven years. Nondiabetic. Modified Mallory's aniline blue stain.

staining is always necessary to identify the cell types as size of both cells and granules varies except within a few small groups of mammals.

Gomori[8] points out the possibility of confusion when the so-called Mankowski cells are present. These are probably degeneration products of acinar cells. They *contain acidophilic granules finer than zymogen granules* but coarser than $\alpha$ granules. Certain stains permit their differentiation.[40] They are perhaps identical with the "onkocytes" mentioned above.

Mitotic figures are occasionally encountered in the island cells of normal pancreases. The occasional presence of mitotic figures in the $\beta$ cells may be regarded as evidence against the transformation of $\alpha$ into $\beta$ cells. (*See below.*)

*Well or even poorly circumscribed islands do not make up all the insular* tissue. With the aid of special stains very small clusters of otherwise ill-defined cells are distinguishable among acini and duct cells.

Masson[43] demonstrated no argentaffin granules in island cells, and O'Leary and Womack[23] point out the importance of this in differentiating island adenomas from intestinal carcinoids.

While a true argentaffin reaction (in the sense of a substance within the *cell which itself has the power of reducing silver without the addition of any* other reducer[7,44]) is not present in the islets, silver impregnation methods may be effectively applied to the islets. This was first shown by Piazza,[45] later by Lasowsky,[46] Nagelschmidt[47] and Ferner.[7] The last-named author has carried out extensive studies with the Gros-Schultze method and on the basis of these has built up a theory regarding the relation between the two main cell types. The essence of Ferner's argument is that the cells which are impregnated by the Gros-Schultze method, including the $\alpha$ cells and various cells scattered among the acini singly or in groups, in the connective tissue and in the walls of the ducts, represent immature ("unreifen") endocrine elements which are capable of forming islet tissue, i.e., $\beta$ cells. In fetal life these "silver cells" are seen in small buds projecting from the ends of the growing glandular formations and as peg-like excrescences from the ducts. Eventually the early islands round off and develop a core of nonimpregnated central $\beta$ cells around which the silver cells are still arranged in layers. In the newborn the silver cells and the islets are much more numerous than in the adult, but according to Ferner this makes no difference in physiological terms since the silver cells are regarded as nonfunctional * resting forms which act merely as precursors of the $\beta$ cells. As evidence in support of this argument he offers an experiment in which long-continued administration of insulin to guinea pigs resulted in an increase in silver cells, reflecting a reversion to the resting stage because of lack of demand for insulin production. He also suggests that the same thing may occur in inanition, citing the case of a young executed woman

* More recently Ferner seems to have modified this view somewhat and to have accepted the hypothesis that the $\alpha$ cells may produce a hormone (*see below*).

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who had apparently been starved in prison and in whom he felt he could demonstrate an increase in silver cells.

Ferner seems uncertain as to the relation of the silver cells to the "clear cells" of Feyrter.[48] Apparently he feels that the clear cells may precede the silver phase. He rejects the idea of Erspamer[49] that the silver cells of the pancreas are essentially identical with the "preentero-chromaffin" cells of the intestine, and points out that there is a fundamental histochemical difference in that the enterochromaffin cells have within their cytoplasm the property of reducing silver salts, whereas in the pancreas the silver cells require the application of an added reducer (such as formalin) to bring out the reaction. Strictly speaking, the term "argentaffin" should be restricted to the former type of cell, *i.e.*, that which is impregnated by the Masson Fontana method without the addition of a reducing agent. (*See* Gomori.[44])

Presumably the silver cells of Ferner are identical with the "muddy" ("truben") *cells which Neubert[4] describes as progenitors of the islets* in the fetus.

Ferner also demonstrated that the $\alpha$ cells would give a positive vitamin C reaction by the method of Giroud and Leblond. The significance of this is obscure; administration of vitamin C to a guinea pig did not appear to cause an increase in these cells. Whether this finding is related to the supposed hyperglycemic hormone of the $\alpha$ cells or to ascorbic acid diabetes is unknown.

Ferner's conclusions are accepted apparently completely and without question by Hess.[50] Others, however, have shown more skepticism. Creutzfeldt[5] and Bargmann and Creutzfeldt[52] have questioned the ability of $\alpha$ cells to turn into $\beta$ cells; they point out that if this were true one would expect to find evidence of it in alloxan diabetes, but, on the contrary, in this condition the $\beta$ cells have been seen to multiply by mitotic division.[51,53] Terbruggen[54,55] notes that mitotic figures may also be observed in the $\beta$ cells of adult humans (*see* also Warren and Root[56]) and that in some islet adenomas the $\beta$ cells appear to spring from nongranular precursors. He prefers to regard the $\alpha$ cell as either a resting phase of the islet cell or as a special cell system. Finally, the wise remark of Gomori[3] may be cited, that if one is to postulate transformation of one of these cell types into another one should be able to find transition forms containing *both* types of granules in the cytoplasm. Using this criterion, Gomori[40] was able to find plenty of evidence for transition forms between D cells and $\alpha$ cells but none for transition between $\alpha$ and $\beta$ cells.

Recently Ferner and Stoeckenius[57] have supplemented Ferner's earlier studies with an investigation of the developing fetal islands, using chiefly the chrome-alum hematoxylin and phloxine method of Gomori. They describe a stage which they call the "mantle island," where there is a peripheral mantle of $\alpha$ cells surrounding a central nucleus of $\beta$ cells with an intervening layer of cells with a homogeneous, nongranular, rose-colored

cytoplasm The latter cells they maintain represent transition forms between $\alpha$ and $\beta$ cells They also describe the $\alpha$ and $\beta$ cell as showing nuclear differences which are very striking in the guinea pig much less so in man Lerner has also written a general review [68] with particular emphasis on the hypothetical glucagon or H G factor supposedly produced by the $\alpha$ cells thus apparently modifying his earlier view noted above that the $\alpha$ cells are nonfunctional immature precursors of the $\beta$ cells

**Transition Theory** The possibility of transition between island and acinar cell is obviously of great importance particularly in the diabetic pancreas However there is now little but historical interest in the question thanks particularly to the masterly studies of Bensley The evidence brought forward by Laguesse[69] and others to support the transition theory faded before the demonstration of specific granules in the island cells Saguchi [9] held that the granules of the $\alpha$ and $\beta$ cells do not take active part in the specific secretion of the island cells but that the F cells with their lipoid corpuscles and urano argentophile apparatus are the most important He further believed[60] the acinar cell may possess an internal secretion and that the acinar cell may change into an island cell with changes not only of the cell body and nucleus but of the intracytoplasmic structures as well Much of his work was based on the frog and has not been confirmed for other species

Otani[6] has also endeavored to revive the old controversy basing his evidence for transition between the islands and the acini not so much upon the specific character of the island cells as on the arrangement of the cells and the relations of the islands to the acini laying special stress on the absence or presence of the capsule

*The existence or nonexistence of a connective-tissue capsule about the* island has been regarded as of importance by many in determining the entity of the island-cell group The Kapsul Frage has been hotly contested However the frequent occurrence of an imperfect connective-tissue capsule is undeniable also its usually incomplete character A complete connective-tissue capsule is usually the first sign of a developing fibrosis

Hartroft[6] notes the frequency with which reduplication of the capillary basement membrane may be found in the diabetic pancreas if connective tissue stains are applied

Otani [5] recognizes three types of island structure

1 Most common islands connected with surrounding acini
2 Islands connected with ducts
3 Least frequent islands strictly separated from the surrounding structures

In the same pancreas he finds wide variations in size shape and relation of the islands In addition he believes[6] that acinar cells are able to change into island cells and that the process of hypertrophy of the islands of

Langerhans in the adult pancreas is mostly due to transformation or hyperplasia of acinar and centro-acinar cells. He does not consider the islands as constituting an independent tissue. He also concludes that new formation of the islands of the adult pancreas may occur by transformation and hyperplasia of acinar, centro-acinar or duct cells. The striking experiments of Ukai[13] have emphasized the independence of the island tissue from the acini and have also shown that the insular epithelium is apparently possessed of greater resistance to injury, if not greater powers of regeneration than is the case with the acinar cells.

Recent authors (e g Gomori[5] Ferner[7] Hard[8] Bargmann[6]) seem to place little emphasis on the possibility of transformation of acinar to islet cells, at least in the adult. Neubert[4] demonstrated the origin of islets from the "end pieces" of the glands in the fetus. Hughes[5] apparently believes that he has demonstrated acino-insular transformation in the rat by graded doses of alloxan, after which the smaller, more sensitive $\beta$ cells are destroyed and replaced from the periphery by *large $\beta$ cells from the acini*, thus pushing the $\alpha$ cells toward the center of the islet and reversing the usual arrangement. Here again one may apply Gomori's criterion and attempt to find cells containing both zymogen and islet granules. Judged by this standard, which of course is not necessarily a valid one, most of the reported work upholding acino-insular transformation must be questioned.

**Blood Supply.**—One of the most striking of the grosser proofs of the autonomy of the islands and one of the most important facts from the standpoint of their function is their peculiar blood supply. Whereas the acinar tissue receives a liberal blood supply from the lobular arteriole with its interacinar capillaries, the islands receive an extraordinarily rich supply and have wide anastomosing sinusoids in almost glomerular arrangement coursing through them. This sinusoidal net is usually fed from one or more arterioles, but sometimes is in direct continuity with the interacinar capillaries. A detailed study of the pattern of the insular circulation is given by Beck and Berg[63] and Beck and Peterson[64] have demonstrated a particularly rapid blood flow through the islands. The capillaries of the islands are thin walled and usually without fibroblastic support. In a case of marked chronic passive congestion shown to one of us (S. W.) by Dr S. B. Wolbach, this rich supply is even better shown than by injection preparations.

**Nerve Supply.**—Between the island cells are the terminations of numerous nerve fibers which form a network around the islands.[65] Gentes[66] detected numerous nerve fibers entering the islands with the blood vessels and distributed along them and between cells. These fibers he felt to be different in number and arrangement from those supplying the acini. Similar conclusions were reached by Ritter.[67] Rarely small ganglia are encountered. Pacinian corpuscles are present in the human pancreas and are very common in that of the cat.

Simard[68] has made a careful study of the so-called neuro-insular com-

plexes in various mammals including man These bodies consist of an intimate association of nervous and epithelial elements and are situated in the inter and intralobular septa They differ in the relative number of epithel al and ganglionic components The epithelial cells are islet cells both $\alpha$ and $\beta$ types Simard seems to think that there is some evidence f r renewal of the e complexes by acino insular transformation He regards them as metasympathetic paraganglia and suggests that they might be chemorecept rs He apparently believes also that the epithelial cells may be transformed into ganglion cells

Excellent general reviews of the cytology of the pancreas have been written by Opie[69] Gomori[3] and Bargmann[6]

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## Chapter 3

# THE PANCREAS IN DIABETES MELLITUS

Few diseases definitely associated with any one function metabolic or otherwise show as wide a range of concomitant pathological change as does diabetes—or as frequent absence of demonstrable pathological change

First the demonstrable pathology in the islands of Langerhans may be considered These changes may be qualitative or quantitative To a pathologist qualitative changes are always more interesting and these will be considered first

### QUALITATIVE VARIATIONS IN THE ISLANDS OF LANGERHANS

[illegible]

the islands are as yet in controversy In the excellent review by Opie [2] one viewpoint is stated as favoring an epithelial and hence intracellular origin for this hyaline substance while the other leans toward a mesoblastic intercellular origin Weichselbaum[3] believed the hyaline change to be secondary to fibrosis and a result of fibroblastic activity Mallory[4] in a few instances succeeded in obtaining from it typical staining reactions for amyloid and others have confirmed this (*see below*)

Obviously it is important to know whether the island cells are merely being replaced by hyaline derived from their own substance after succumbing to some injury or whether they are being strangled by gradually increasing deposits of hyaline laid down in and about the stroma of the islands Or in cruder form is hyalinization of the islands the result or the cause of diabetes in those cases in which it is present?

If this hyaline were epithelial in origin and a product of degeneration of island cells we should find it as commonly in young diabetics as in old and we never should expect to find it in the absence of diabetes However of 200 cases of hyalinization of the islands in diabetes studied by one of us (S W ) 97 per cent occurred in individuals over forty years of age At this age hyaline changes begin to be noted in the smaller blood vessels particularly those of the spleen an organ which incidentally lies in almost the same vascular circuit as does the pancreas

In spite of this preponderance of hyalinization of the islands in the older age groups curiously enough the first case to be described (Opie[1]) showing

this lesion was that of a girl aged seventeen years whose diabetes began two years before death

One of the arguments brought forward in favor of the epithelial origin for hyaline in the islands is that with Mallory's aniline blue stain certain of the island cells, assumed to be those which later will be replaced by hyaline, stain intensely blue, and that later masses of hyaline appear in them which ultimately fuse, forming large masses about the capillaries. One of these cells is shown in Figure 3. In many cases of diabetes examined

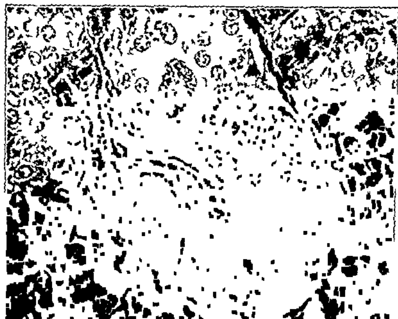


Fig 3 Island showing large degenerating cell No 3015 Female aged forty-eight years Duration of diabetes three years Mallory's aniline blue stain × 560

in this laboratory bluish staining cells similar to those described can be seen, provided the tissue is fresh and the fixation satisfactory. As a rule they are somewhat irregularly distributed through the substance of the islands. At other points small masses of hyaline can be seen. However, similar blue-stained cells can be found in the islands of nondiabetic individuals and in islands which show no trace of hyalinization. The blue-stained cells occur scattered throughout the island and do not show the definite relationship to the blood vessels that the hyaline does. These cells have been named D cells by Bloom[5]

Although admittedly unreliable, the staining reactions of this hyaline throw some further light on its nature. In the first place, out of 31 cases of hyalinization of the islands suitably fixed, following the suggestion of Dr F B Mallory, one of us (S W) was able to demonstrate a positive

PLATE II



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amyloid reaction with methyl violet or iodine green in 14 Recently several investigators have given considerable attention to this point Gellerstedt,[6] chiefly on the basis of studies with the methyl violet reaction concluded that the hyaline is actually amyloid and that the condition should be called insular (para)amyloidosis' He holds that the peculiar localization in the islets is to be regarded as one manifestation of so called senile amyloid deposition which he looks upon as a common phenomenon stating that careful search will reveal amyloid in the tissues of around 45 per cent of males over 50 years of age Van Beek[7] made similar observations Arey[8] and Ahronheim[9] also hold that the hyaline found in the islands of diabetics is actually" or "in all probability amyloid basing their conclusions on the usual staining reactions The fact that the hyaline is deposited in contact with the walls of blood vessels in the islands as noted in previous editions of this book, is emphasized by Arey,[8] who found hyaline in the pancreases of 16 6 per cent of nondiabetics and 71 7 per cent of diabetics over fifty years of age He states that the hyaline of the islets may not be identical with the amyloid in the liver and spleen in cases of generalized amyloidosis since it gives a somewhat lighter stain than the usual amyloid with methyl violet hematoxylin and eosin and the Mallory-Heidenhain method Ahronheim[9] found the hyaline more abundant in the younger obese group of diabetics He obtained positive reactions in 67 of 105 diabetics and in 5 of 50 nondiabetics of similar age Many of his patients in both categories had hypertension He emphasized that amyloidosis of the islands is an isolated feature not usually associated with amyloid in other tissues

The entire concept of atypical amyloid or paramyloid is an exceedingly vague one[10-14] For instance Bauer and Kuzma[13] state The term paramyloid signifies the atypical distribution and variable staining reaction of a hyalin like material Such a definition obviously allows for the inclusion of a variety of substances of different chemical nature

King[10] points out that it is illogical to classify amyloid as primary and ' secondary and prefers the term typical and atypical basing his classification on anatomic distribution of the deposit He also describes a simple silver stain Amyloid may well be a protein of variable composition the various stains reacting with different prosthetic groups[5 16 17]

Perhaps as suggested by one of us (S W[18]) atypical amyloidosis is a reflection of dysfunction of fibroblasts Jones and Frazier[19] found an intimate relation between reticulum fibers and amyloid deposits They note also that amyloid stains red with leukofuchsin and suggest that it may represent a *mucopolysaccharide combined* with a variety of different proteins (including those of reticulum as well as those involved in antigen antibody reactions) (*See* Chapter 28 for further discussion of this concept )

Certainly amyloid is frequently associated with hyperglobulinemia (Teilum[11]) but to assume that it is simply excess globulin deposited from the blood stream would seem unwarranted in the present state of our knowl

edge Indeed it seems quite arbitrary to include as Teilum[11] does cases in which the usual methyl violet and Congo red reactions are negative A classification without histochemical basis would appear to be open to question

Since the chemical nature of atypical amyloid or paramyloid if it be an entity is unknown[16,17] and the substance is defined by certain nonspecific tinctorial reactions and since these reactions are found inconstantly in hyalinized islands of Langerhans it would seem wise to withold judgment Labeling the hyaline material amyloid does not solve any problems but rather raises new ones and until its chemical nature is better defined it is perhaps preferable to refer to it by the usual term of hyaline Indeed it might prove more fruitful to emphasize the polysaccharide component (*See* Chapter 28 )

The hyaline ordinarily gives the following staining reactions

Mallory s aniline blue—translucent blue
Phosphotungstic acid hematoxylin—brown
Phosphomolybdic acid hematoxylin—deep blue
Eosin methylene blue—pink
Masson s iron hematoxylin light green—bright green
Iron hematoxylin Van Gieson—yellowish
Best s carmine stain—pink to old rose
Periodic acid-Schiff reagent—pale pink

The staining reactions obtained from hyaline about the arterioles of the spleen from several cases which also showed hyalinization of the island of Langerhans were identical Occasionally a slight fibrillar structure can be demonstrated in the hyaline

Study by the serial section method shows that the masses of hyaline are always in contact with the walls of the vessels of the islands and always intercellular Masses that are apparently isolated can always be traced in the course of one or more sections 3 microns thick to the perivascular deposit of which they are a portion

Should hyaline be the result of cellular degeneration secondary to diabetes it ought never to be present in the absence of diabetes However cases have been reported by Cecil[18] Wright[19] Arey[4] Ahronheim[2] and others where hyalinization of the islands was present in nondiabetics It is true that in a few of these cases objections can be raised on the ground that the individual might be in reality diabetic but owing to terminal malnutrition failed to show signs of diabetes just as he might fail to show these signs under rigid dietary treatment But not all these cases can be thus disposed of

Moreover of 4 cases in our series where fairly complete clinical data have been available to rule out the occurrence of diabetes mellitus hyaline has been present in the islands to a moderate degree in 3 cases and to a marked degree in one Studies of deposition of hyaline in island cell adenomas[20]

where hypoglycemia rather than diabetes exists further confirm this viewpoint. Meissner[91] has found hyaline quite constantly in functional adenomas but not in the nonfunctional ones (Chapter 22) and suggests that it may be related to the secretion of insulin rather than representing a degeneration.

Hyaline degeneration is most common in older diabetics, as has already been said, and in mild diabetics. In our material 6 per cent of the cases

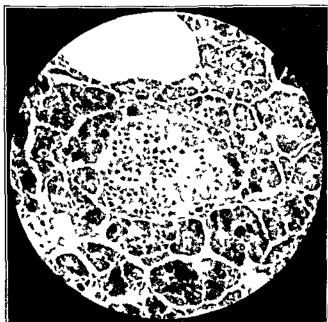
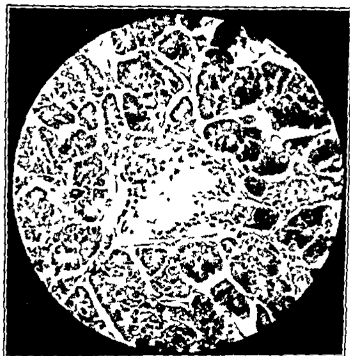


Fig. 4.—Early hyalinization of island of Langerhans. M077. Male, age sixty-six years. Duration of diabetes fourteen years. Eosin methylene blue stain. × 170

through forty years of age showed hyaline, as against 45 per cent of those over forty years of age. In carefully studied cases definitely known to have had diabetes over ten years, 50 per cent showed hyalinization of the islands of Langerhans.

A striking feature is the unequal involvement of the islands in the same pancreas, some being converted to completely hyalinized masses with only a few fibroblasts and capillary endothelial cells present, while others may be completely spared. Still others show varying degrees of transition between these two extremes. This seems to point to either striking inequalities of involvement or to the replacement of destroyed islands by new ones.

Frequently the insular epithelium does not tamely submit to the hyaline invasion, but as the hyaline separates the cells from their blood supply and



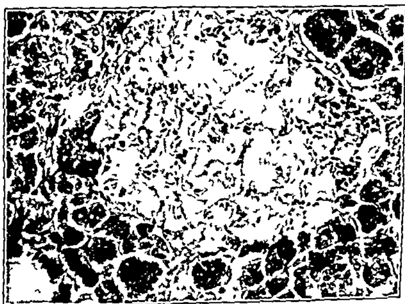
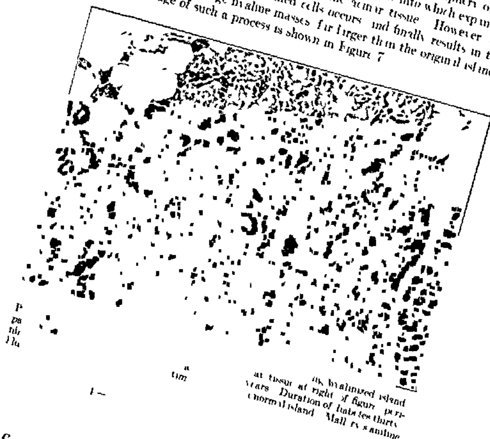


Fig. 6. Marked hyalinization of island of Langerhans. DA2[illegible]0. Female aged sixty years. Duration of diabetes five years. Note the few epithelial cells persisting at right hand margin of island. Mallory's aniline blue stain.

finally forces them out of existence growth occurs at the periphery of the island particularly if adjacent to fat or fibrous tissue into which expansion can take place more readily than into the acinar tissue However encroachment on these newly formed cells occurs and finally results in the formation of very large hyaline masses far larger than the original island The end stage of such a process is shown in Figure 7



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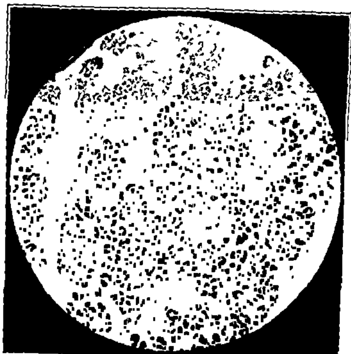


Fig. 8.—Pancreas showing large numbers of hyaline islands. U24-1. Female aged forty-nine years. Duration of diabetes fifteen years.

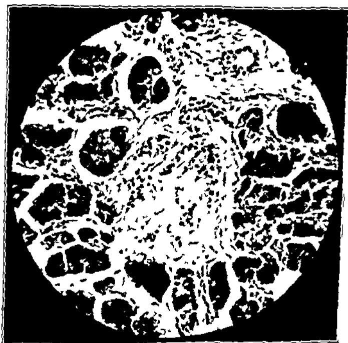


Fig. 9.—Extreme hyalinization of island of Langerhans. Note blood cells in still patent capillaries. M97). Male, aged sixty-six years. Diabetes of fourteen years' duration. Phosphotungstic acid hematoxylin stain. × 170.

Rarely the hyaline becomes the seat of calcium deposition so that the pancreas may be studded with minute calcified nodules One such case is described by Mallory [4] and another by Gibb and Logan [20] Escudero [3] states that calcification is extremely rare

The most remarkable case of calcification of the islands is that of Fischer [21] The patient, an eighteen year-old boy, died in coma after typical juvenile diabetes The pancreas weighed 100 grams was somewhat firm and its cut surface revealed many minute white spots These proved to be calcified hyalinized islands Many of the islands were large and they were very numerous Fibrosis as well as hyalinization was present While the pancreas was somewhat fibrotic, the acini were not damaged

Hyalinization of the islands of Langerhans in light of the foregoing evidence may be considered as due either to amyloid ( paramyloid ) or to production of intercellular substance by fibroblasts and possibly by endothelial cells When the process becomes sufficiently marked to destroy a number of the epithelial cells or to separate them from the blood stream by a practically impermeable membrane diabetes will develop It is of no slight interest that this pathological product usually appears in the later years of life about the time that marked hyaline thickening of the small arterioles is prone to occur The reason for the specific involvement of the islands rather than the acinar tissue is difficult to interpret although the rich vascularity of the islands may provide a clue

TABLE 5 — LESIONS IN THE ISLANDS OF LANGERHANS IN 811 DIABETIC PANCREASES

| Age | Cases | Normal | S+ | S++ | S+++ | H+ | H++ | H+++ | Hypertrophy | Hydropic change | Pyknotic nuclei | Hemorrhage | Adenoma | Lymphocytic infiltration |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 0 10 | 13 | 1 | | 1 | | 1 | | 1 | | 7 | 4 | | | 3 |
| 11 20 | 15 | 17 | 6 | | | 1 | | | | 1 | | | | 1 |
| 21 30 | 41 | 20 | 5 | | 2 | | 1 | | 3 | 4 | | | | |
| 31 40 | 28 | 16 | 7 | 8 | | | 1 | | | 4 | | 1 | | |
| 41 50 | 85 | 30 | 11 | 10 | 1 | 4 | 11 | 11 | 6 | 4 | | 1 | | |
| 51 60 | 106 | 64 | 27 | 17 | 6 | 2 | 11 | 6 | 15 | 6 | 4 | | | 2 |
| 61 70 | 254 | 70 | 27 | 16 | 4 | 30 | 56 | 50 | 25 | 6 | 4 | | 7 | |
| 71 80 | 131 | 47 | 22 | 8 | 4 | 20 | 15 | 37 | 31 | 5 | 4 | 1 | 1 | 1 |
| 81+ | 14 | 4 | | 1 | | 2 | 1 | 4 | 1 | | 1 | | | |
| • | 4 | 2 | | 1 | 1 | | | 1 | | | 1 | | | |
| Total | 811 | 271 | 105 | 63 | 18 | 90 | 119 | 125 | 63 | 32 | 20 | 3 | 6 | 11 |

S = fibrosis or sclerosis H = hyaline
Totals apparently do not check because of some coexisting lesions.

Through the courtesy of Dr. W. L. [illegible] one of us (S. W.) was able to examine the pancreas of a diabetic infant of [illegible] months. It showed a remarkable picture of polymorphonuclear leukocytes in many islands, together with some lymphocytes and fibrosis of at least one island.

Von Meyenburg[illegible] calls the picture of lymphocytic infiltration "insulitis" and attributes it to overstrain. One wonders about the possible presence of unrecognized mumps in some of these cases.

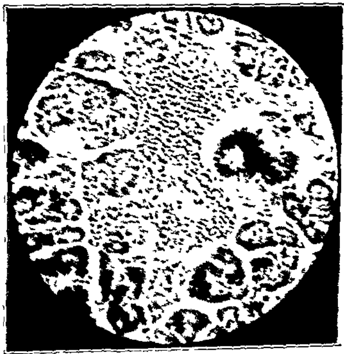


FIG. 11. Marked lymphocytic infiltration and slight fibrosis [illegible] island of Langerhans. M612. Male aged [illegible] years. Duration of diabetes eleven months. [illegible] × 170

**Hydropic Change.** One of the diabetic lesions most difficult of comprehension from the pathological standpoint is hydropic change, described by Weichselbaum[illegible] and further emphasized by Allen.[illegible] On the [illegible] of experimental work and of [illegible] observations Allen[illegible] regarded [illegible] as of much importance on the follow[illegible] [illegible] [illegible] diagn[illegible]

"(A) Its presence at [illegible]

diabetes. [illegible] dia.

"(B) It emphasizes the pro[illegible]

"(C) It adds to the evide[illegible]

and clinical diabetes.

"(D) It explains the p[illegible]

consequent upon excessive [illegible]

(E) From a broader physiological standpoint it offers the only proved example of anatomical breakdown of cells due to overstimulation of an internal secretory function

There has been no question from the early experiments of Allen[31] and of Homans[3] to the present time that hydropic change is an important lesion in experimental diabetes (*See* Chapter 25) There are so many factors to be considered in human diabetic material however that its interpretation is more questionable there

The striking feature of hydropic change is that it appears to be not the result of injury but of excessive functional strain Most of the intracellular reactions to injury short of necrosis are represented by the liberation of fatty or albuminous and related substances from the protoplasm giving the pictures commonly described as fatty degeneration albuminous de-

[illegible]

until the cytoplasm of the cell is apparently replaced by a watery fluid leaving the nucleus alone in the embrace of the distended cell membrane Apparently this change is reversible in its slighter degrees but once fully established leads to the atrophy and disappearance of the cell involved If the work of Toreson (*see next section below*) is confirmed and it is found that the hydropic appearance of the cell is actually due to glycogen then much of the difficulty in the concept of reversibility of the lesion will disappear

It is striking that this response to functional overstrain is unique among the endocrine glands and indeed among all the various organs Perhaps the simplest physiological analogy to the pancreas in diabetes would be the thyroid in cases of myxedema yet in the thyroid cells no such anatomical change develops

It is rather difficult to explain all cases of hydropic change on the basis of functional overstrain of the $\beta$ cells In going over the earlier series of diabetic pancreases one of us (S W) was struck by the frequent occurrence of appearances suggesting hydropic change in many of the island cells of those pancreases removed over three hours post mortem and the comparative rarity of this finding in pancreases removed one hour or less post mortem Allen[33] believes that hydropic degeneration is demonstrable in the human pancreas whenever diabetic symptoms have been sufficiently intense and prolonged but is ordinarily missed in the mildest cases He also emphasized the importance of fresh tissue for its demonstration However as pointed out by Conroy [34] it is difficult to see how a cell with cytoplasm practically replaced by vacuolization or by one large vacuole could through post mortem change acquire normal-appearing cytoplasm

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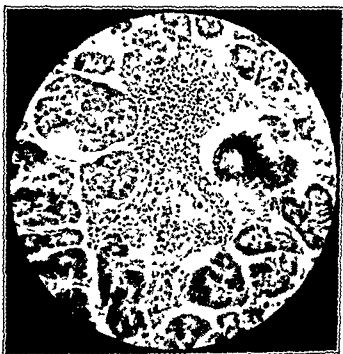


Fig 11 —Marked lymphocytic infiltration and slight fibrosis replacing island of Langerhans M932 Male aged nineteen years Duration of diabetes eleven months Eosin methylene blue stain × 170

**Hydropic Change** —One of the diabetic lesions most difficult of comprehension from the pathological standpoint is hydropic change, described by Weichselbaum,[29] and further emphasized by Allen[30] On the basis of experimental work and clinical observations Allen[30] regarded this lesion as of much importance on the following grounds

"(A) Its presence affords a positive microscopic diagnosis of active diabetes

"(B) It completes the proof of the island theory of diabetes

"(C) It adds to the evidence of the essential identity of experimental and clinical diabetes

"(D) It explains the permanent lowering of assimilation in diabetes consequent upon excessive diets

(E) From a broader physiological standpoint it offers the only proved example of anatomical breakdown of cells due to overstimulation of an internal secretory function

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The typical hydropic change is totally different from these The specific granules disappear and are replaced by vacuoles, which grow and coalesce until the cytoplasm of the cell is apparently replaced by a watery fluid leaving the nucleus alone in the embrace of the distended cell membrane Apparently this change is reversible in its slighter degrees, but once fully established, leads to the atrophy and disappearance of the cell involved If the work of Toreson (*see next section, below*) is confirmed and it is found that the hydropic appearance of the cell is actually due to glycogen, then much of the difficulty in the concept of reversibility of the lesion will disappear

It is striking that this response to functional overstrain is unique among the endocrine glands and, indeed, among all the various organs Perhaps the simplest physiological analogy to the pancreas in diabetes would be the thyroid in cases of myxedema yet in the thyroid cells no such anatomical change develops

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Allen[33] also considers that the pathology of diabetes indicates "the diabetic pancreas represents in most cases a burned-out conflagration and the

chief or sole cause of aggravation of the condition lies in hydropic degeneration of the islands

The relation to duration of the diabetes is somewhat uncertain but the most striking cases of hydropic change which we have seen have been in *fulminating* cases of diabetes of relatively short duration Dr John R Williams gave the privilege of studying the pancreas of one case of only three weeks duration which showed marked hydropic change Several of the early cases of diabetic children with severe diabetes progressing rapidly to coma showed suggestive changes but post mortem change could not be ruled out



Fig 19 Pancreas showing apparent hydropic degeneration Note extreme vacu... This appearance wa... 86 Four hours po... ...odpa ture s pol chrom...

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It may be objected that the rarity of hydropic change in this series (onl 32 cases) is due to the large number of insulin treated cases on the theor that insulin by reducing functional strain reduces the frequency of th les on

If Allen s statement holds that the chief cause of aggravation of diabete lies in hydropic degeneration of the islands this is of great importance fo f the above interpretation can be put on observations from this laborator

Fig. 13—So-called hydropic change in island of Langerhans in rabbit made hyperglycemic with large doses of cortisone. The vacuolated cells were shown to contain glycogen (*see* Figure 14) × 650



if we accept functional overstrain as the cause of this change and especially if the appearance is actually due to the presence of glycogen (see be[low]). Assigning to hydropic change such an important role and finding it al[so] in insulin treated cases the implication is that such cases should not [have] tolerance. However the change is nearly as rare in cases not given insulin (some dying before the Banting Era some during it) as in those treated with insulin.

We have seen pancreases from pre insulin cases of diabetes of sh[ort] duration dying in coma and pancreases from mild diabetics dying fr[om] arteriosclerosis or intercurrent infection after twenty five or thirty ye[ars] of diabetes and neither group showing evidence of hydropic change. T[wo] cases of apparent hydropic change were encountered in the control seri[es] and the histories of both when carefully studied gave no evidence [of] diabetes.

In no case of the series reported by Root and Warren[b] was hydrop[ic] change positively diagnosed. Conroy[34] before the days of insulin foun[d] hydropic change in only 1 of 12 diabetic pancreases exhaustively studied.

While the existence of hydropic change as a clear-cut lesion in experimental animals and human diabetics is unquestionable nevertheless it can be very closely simulated by post mortem change. Such a case is well shown in Fig. 12. Several times one of us (S. W.) has succeeded in producing apparent hydropic change by the simple expedient of leaving a portion of normal pancreas at room temperature for eight or ten hours before fixation. The lapse of many hours between death and autopsy in most of Weichselbaum's cases may have some bearing on the frequency with which he found the lesion.

**Glycogen** —It is possible that the entire concept of hydropic change in the islands of Langerhans may have to be revised in the light of the new work of Toreson[36] which will require extensive study and confirmation.

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LEGEND FOR PLATE III

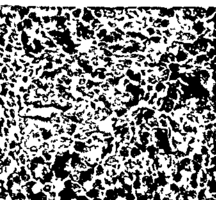
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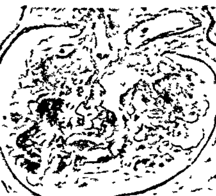
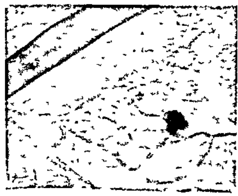
*C* Island of Langerhans of normal rabbit. Gomori's chrome alum hematoxylin and phloxine stain.

*D* Island of Langerhans from rabbit made permanently diabetic with alloxan. It consists entirely of α cells with the possible exception of three or four hydropic "β cells"

PLATE III





This investigator has shown that in at least some instances vacuolation of the cytoplasm of the cells of the islands is associated with the deposition of glycogen. His results are particularly striking in experimental animals. Through the courtesy of Dr. Toreson we have had the privilege of examining some of his beautiful preparations showing vaculoated cells of the islands loaded with glycogen in a dog made diabetic with anterior pituitary extract, in a rabbit given alloxan, and in a rabbit treated with cortisone. The glycogen deposits are massive, are demonstrable by both the Best carmine and periodic acid-Schiff reagent methods, and can hardly be attributed to artifact. We have been able to confirm his findings in a rabbit given massive doses of cortisone (Figs. 13 and 14). In Plate III there is illustrated an example of hydropic change apparently due to glycogen as indicated by the failure to stain with the PAS method after treatment with diastase.

In human material the results are less convincing, perhaps due in part to post mortem hydrolysis of the glycogen. In the preparations which Dr. Toreson sent, and in some of our own cases, fine granules of glycogen can be demonstrated (Plate III) which are removed if the slide is previously subjected to the action of diastase or saliva. However, it is not always clear that these granules are associated with vacuolation of the cells. Probably further work will need to be done to establish whether the microscopic appearance which has been called hydropic degeneration in the past is actually due to the presence of glycogen or whether glycogen may not be deposited, with or without vacuolation, in the cells of the islands as a part of the general disturbance of carbohydrate metabolism in diabetes, in which case it would be analogous to the similar deposits in the renal tubules, nuclei of liver cells, and iris epithelium (*see* Chapter 5). If Toreson's work is confirmed, another point which will require clarification will be the question of why previous investigators who studied hydropic change with great thoroughness (*e.g.*, Allen) did not encounter glycogen. One of us (S. W.) has in the past done many stains for glycogen, successfully demonstrating this substance in the duct epithelium, but not being impressed with its presence in the islands.

If Toreson's conclusions are indeed correct, their theoretical implications will be considerable. The whole question of whether hydropic change does indeed represent injury or functional overstrain of the islands, as has always seemed to be true, will have to be re-examined. If it does represent damage to the cells, the situation is a unique one; there is no evidence, as far as we are aware, *that the long-continued presence of glycogen in the* renal epithelium, liver cells, or iris epithelium of the eye exerts any injurious effect whatever. Injured cells may become vacuolated due to the presence of fat or fluid, but glycogen is usually not found under such conditions.

If one accepts the usual view, outlined in the previous section (and in Chapter 25), that hydropic change does indeed represent the earliest visible

evidence of injury to the cells and if one assumes that the presence of glycogen is the explanation for the vacuolation then one is led to wonder wh

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betes as well (*see* Chapters 25 and 26) An adequate explanation would be provided for the experimental production of hydropic change by massive doses of glucose[37] and for the observations of Gomori[38] who noted vacuolation of the $\beta$ cells in surgical biopsies of the pancreas from patients who had had large amounts of glucose pre-operatively The presence of glycogen in the vacuoles would also of course explain quite well the puzzling reversibility of the lesion in the experimental animal and apparently in man

Much remains to be done in relation to Toreson s fascinating observations If for example it could be shown that so-called hydropic change is actually related to the level of the blood sugar as is the vacuolation of the renal epithelium many of the puzzling considerations relating to severity and duration of diabetes effect of insulin and the like discussed above would disappear Actually hyperglycemia may not be the important factor as is suggested by the work of Duff and Toreson [39] who were able to prevent or reverse the hydropic change in alloxan diabetes of rabbits by giving small doses of insulin even in the presence of sustained hyperglycemia

It is of interest that Medwedeff[40] described glycogen in the islets in a single case of diabetes in 1930

**Pyknotic Nuclei** —One appearance in the islands which is interpreted only with considerable difficulty is the presence of pyknotic nuclei and somewhat shrunken cells Occasionally islands are found closely resembling normal ones except for the presence of extreme pyknosis of the nuclei and acidophilic cytoplasm This change was encountered in 6 cases These cases ordinarily show no other lesion in the islands, if indeed this nuclear change may be considered a lesion One cannot help but feel that it may be either post mortem change or improper fixation that produces these shrunken extremely dense nuclei However cases autopsied soon after death and showing no evidence of post mortem change do show these pyknotic nuclei in the island cells Gibb and Logan[22] encountered these islands in 15 cases among 142

**Necrosis** —Frank necrosis of the islands is very rarely found at autopsy It may occur as a complication of infectious disease but is seen in most striking form as a result of injection of alloxan either in the experimental animal or in the few cases in which the drug has been used in man The necrosis induced by alloxan is amazing not only for its specificity for the $\beta$ cells of the islands but also for the fact that the process of cell death goes on in the almost complete absence of any cellular reaction in the form of leukocytes

**Hypertrophy**.—Hypertrophy of the islands is not infrequently encountered. Cecil[41,42] has pointed out two types of hypertrophy of the islands—one a simple increase in size of the island without change in the character of the individual cells, the other a change in the cells to a columnar type with the nuclei located centrally. Rudimentary islands of this latter type Weichselbaum[43] encountered in 19 out of 151 cases of diabetes and regarded them as *representing regeneration of islands from duct epithelium*. Cecil agreed that this type of hypertrophy is seen in islands newly formed from duct epithelium. He found 34 cases of hypertrophy and

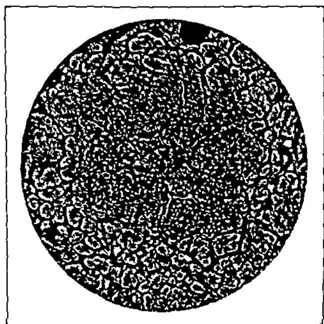


Fig. 18.—Hypertrophy of island of Langerhans. A09-C. Non-diabetic. Eosin methylene blue stain. × 40.

probable regeneration in 100 diabetic autopsies, none in 33 cases of chronic pancreatitis and only 1 in 17 cases of carcinomatous involvement of the pancreas. MacCallum[44] gives an excellent description also.

The two types of hypertrophy are easily distinguished. The coiling snake-like columns of cells in the islands in the columnar type are quite different in appearance from the typical sinusoidal structure, although the close relation of cells and vessels is maintained. The epithelial cells themselves are somewhat changed, tending toward a columnar rather than the usual cuboidal polyhedral shape. Irregular projections among the acini at the periphery of the island are common. This change appears to have no definite correlation with the pathological physiology.

Hypertrophy as well as hyperplasia of the islands is seen commonly in the infants of diabetic mothers (*see* Chapter 23)

Ogilvie,[45] using the method described previously by him (*see* Chapter 2) has more recently made a special study of hypertrophy of the islands He finds enlarged islands particularly in obese persons and ascribes them to a pancreotropic factor of the pituitary (*See* page 22 for further references on the volume or total mass of island tissue )

On occasion it may be difficult to distinguish large islands from small adenomata [21] The adenoma shown in Figure 17 if it had a less well-defined capsule and did not exhibit so much compression of the surrounding tissue might be regarded as an unusually large island

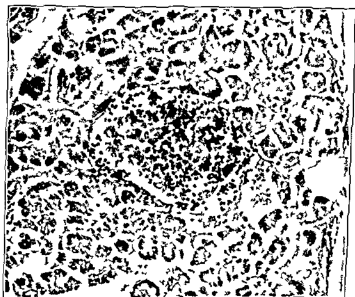


FIG 16 —Post mortem change simulating hemorrhage into island of Langerhans No 1638 Male aged sixty-eight years Nondiabetic

Thirty-eight cases in the earlier series showed a greater or less degree of hypertrophy of the islands of Langerhans Usually not all the islands are hypertrophied but only a moderate proportion of them However occasionally the majority may appear much enlarged Islands were considered hypertrophic when they measured more than 400 micra in diameter There is no correlation between the occurrence of hypertrophy the age of the patient the duration of the diabetes or [illegible]erity of the diabetes In one instance markedly hypertrophic [illegible] present in the tail of a pancreas from w[illegible] [illegible]tically all [illegible]ppeared secondary to carcinoma of [illegible] [illegible]e pancreas [illegible]d developed ten years before death [illegible] controlled [illegible]ven years Then the

case became more or less insulin resistant and symptoms referable to the tumor became increasingly prominent

Herxheimer[46] by ligating the pancreatic duct of chickens produced a marked increase in island tissue and a fivefold increase of insulin content. As this occurred the acinar tissue atrophied

Huttl[47] noted large islands at autopsy in 3 diabetic patients on whom he had performed pancreatic ligation by the Mansfeld technique

**Hemorrhage** —Hemorrhage into the islands has been described but is probably either a post mortem change or an artifact as a glance at Figure

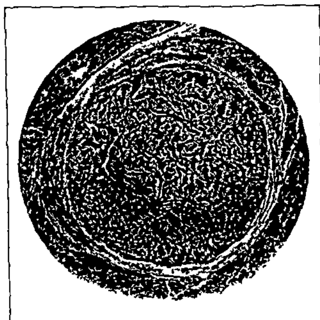


Fig 17 —Small adenoma of island of Langerhans. Note capsule and compression of surrounding tissue. H26–1[illegible] Female aged fifty three years. Nondiabetic. Mallory's aniline blue stain × 40

16 suggests. In only 2 cases was this hemorrhagic change at all noticeable and in one of these, a man aged seventy two years with diabetes of twenty two years duration, cardiac decompensation was the cause of death with extreme passive congestion

**Adenoma** — Adenoma of the islands of Langerhans was encountered only three times in this series

Among the series of adenomas of the islands of Langerhans reviewed by one of us[48] there was only one, that of Heiberg[49] which occurred in a diabetic. Here there was a large mass of insular tissue 6 by 5 mm in diameter,

which was found in the pancreas of a white female aged sixty four years dying in diabetic coma.

In one of the cases reported by Smith and Seibel[9] the patient showed diabetic symptoms during an attack of lobar pneumonia prior to death.

Buchner[51] reported an insular adenoma in a male aged fifty seven years whose diabetes present for two years terminated in hypoglycemic coma and death following injection of a small amount of insulin.

The association of insular adenoma and diabetes is apparently a rare one and the earlier cases were reported before the description of the newer differential stains for the islands. That the use of these stains is important

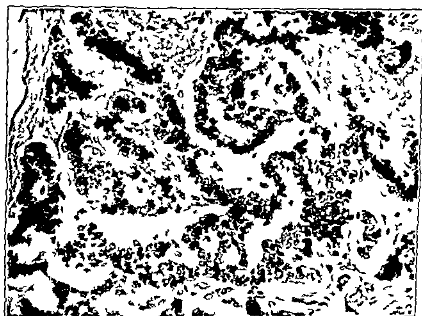


Fig 18 Adenoma of island of Langerhans A09 101 Female aged forty-eight years Nondiabetic Mallory's aniline blue stain × 200

is suggested by the case described by Hess[5] of a non insulin producing carcinoma of islets. When studied with the modified Gros-Schultze method of Ferner the great majority of cells making up the tumor were silver positive and therefore presumably α cells. The importance of such a finding in relation to the recent work on isolation of a hyperglycemic factor from the α cells is obvious. Certainly in the future it will be desirable to study all such cases with the newer staining methods.[52] In addition to the Gros-Schultze method the latest techniques of Gomori and Terbruggen may be recommended (*see* Appendix A).

**Islands Apparently Normal** — *Of particular importance* from the theoretical as well as practical standpoint is the fact that a considerable number

of diabetic persons show no demonstrable pathology in the islands of Langerhans when studied by the usual methods. Out of 811 pancreases available for detailed study in this series 271 or 33 per cent, showed islands which were normal so far as could be determined by ordinary histological means. A considerable proportion of these normal islands occurred in insulin treated cases that possibly might have shown hydropic change if they had not been so treated. However a very fair number occurred in preinsulin days, or in cases which had received no or inadequate insulin. Also the great majority occurred before the newer differential stains of Lerner, Gomori and others were available. No diabetic pancreas can now be said to have normal islands unless studied intensively with these new techniques, preferably also with some attempt to estimate the total mass of insular tissue (*see below*). However there seems to be no question that such cases do exist.

The existence of these normal islands in diabetes mellitus has long been a puzzle to pathologists. It is rather difficult to conceive how we can have an insular diabetes with normal islands of Langerhans present, particularly in a disease that persists as long as life itself. This leads to one of four hypotheses: (1) the islands may be normal and insulin may be produced but it is either defective in quality or neutralized by some substance in the body; (2) the islands may be normal but their secretory activity inhibited; (3) the diabetes may be of other than insular origin; (4) the insulin may be formed normally by normal islands but through failure in transport the insulin is not brought into the blood stream and so not distributed to where its effects are needed. Whichever (if any) one of these hypotheses is true (No. 3 seems to be currently favored) we must face the fact that cases of diabetes absolutely identical with one another from the clinical standpoint show no evidence of structural disease in the islands of Langerhans in one instance and show marked injury of various types in others.

It remains for further investigation to establish how large is the group with normal islands when studied by *all available methods*, both qualitative and quantitative (*see* page 62). Until that time suggestions regarding the etiological significance of such factors as other endocrine glands or the liver must remain partly speculative.

Cham[24] emphasizes the multiplicity and variability of the lesions that may be encountered in the pancreas in diabetes and points out that careful study must be undertaken before a pancreas is classified as "normal."

**Fatty Infiltration.** One minor change concerning the importance of which there has been a good deal of discussion is the presence of fat in the island cells. This was first pointed out by Dogiel[55]. In many cases of diabetes this can be demonstrated by either osmic acid or Sudan III or IV in many of the island cells, usually in considerably greater degree in the islands than in the acinar tissue. In sections stained with Sudan IV the islands may appear under low magnification as definitely orange spots. At one time this was considered to be a specific change characteristic of dia-

betes Weichselbaum and Stangl[56] found in their material that diabetics showed more fat in the islands of Langerhans at autopsy than did those dying at the same ages from other disease conditions. The work of Summers[57] showed that the presence of fat in the islands was not characteristic of diabetes but was present in other diseases notably alcoholic intoxication in which it was particularly prominent In fact Summers felt the

quent in diabetic than in nondiabetic persons was probably a result rather than a cause of diabetes In its most striking form the islands



Fig 19 —Pancreas showing lipomatosis and moderate fatty infiltration of islet epithelium D420-35 Female aged fifty four years Duration of diabetes five years Sudan IV stain × 310

appear in Sudan stained sections as deep orange-red spots Wilder found marked fatty infiltration of 11 cases moderate in 15 Table 6 shows the degree of fatty infiltration in a number of pancreases from our series both diabetic and nondiabetic

TABLE 6 —FAT IN THE ISLANDS OF LANGERHANS

| | Degree of infiltration | | |
|---|---|---|---|
| | Slight | Moderate | Severe |
| Diabetic cases | 0 | 18 | 13 |
| Non-diabetic cases | 1 | 2 | 6 |

Apparently there is no significance to be attached to this lesion An example of moderate infiltration is shown in Figure 19

It is interesting to compare fatty change with glycogen infiltration of the islands (*see* page 46) Apparently either substance may or may not be associated with vacuolation of the cytoplasm

TABLE 7 —QUALITATIVE INSULAR CHANGES IN DIABETES MELLITUS

| Author | Total cases | Normal | Hyaline | Fibrosis | Hydropic change | Pyknotic nuclei | Hemochromatosis | Lymphocytic infiltration | Hypertrophy |
|---|---|---|---|---|---|---|---|---|---|
| Cecil | 90 | 0 | [illegible] | 49 | 0 | 0 | [illegible] | 1 | 41 |
| Wilder[54]* | 29 | 8 | 4 | 12 | 0 | 0 | 0 | 5 | 0 |
| Gibb and Logan[55] | 142 | 11 | 30 | 79 | 0 | 15 | 7 | 0 | 0 |
| Herxheimer[56] | 97 | [illegible] | 38 | 13 | 0 | 0 | 0 | 0 | 0 |
| Warren and LeCompte | 811 | 271 | 333 | 186 | 32 | 20 | 16 | 11 | 63 |
| Total | 1169 | 312 | 437 | 339 | 32 | 35 | 25 | 17 | 104 |

* Only those of Wilder's cases are included in which the pancreas was specifically described

## QUANTITATIVE VARIATIONS IN THE ISLANDS OF LANGERHANS

Enumerating the islands in the human pancreas is extremely difficult and for practical purposes only rough estimates are possible based on the total number of islands in a number of representative sections taken from different portions of the gland Any method such as this of course is exposed to numerous errors and presumably the statements as to the number of islands occurring in a given human pancreas are all open to question The methods worked out by Bensley[60] and his students for enumeration of the islands in experimental animals are extremely valuable there but are rarely applicable to human tissues Clark[61] working in Bensley's laboratory was able to count fairly accurately the number of islands in the pancreas of seven human adults He found the number to range from 120 000 to 1 760 000 As noted in Chapter 2 Ogilvie found values in infants ranging from 117 226 to 2,325 123 and Bargmann[62] records counts in 5 adults varying from 208 369 to 1 760 000 (*See* Chapter 2 for Ogilvie's data on the weight of the normal island tissue )

betes Weichselbaum and Stangl[56] found in their material that diabetics showed more fat in the islands of Langerhans at autopsy than did those dying at the same ages from other disease conditions *The work of Symmers*[57] showed that the presence of fat in the islands was not characteristic of diabetes but was present in other diseases notably alcoholic intoxication in which it was particularly prominent In fact Symmers felt the lesion was to some extent characteristic of prolonged alcoholic intoxication

*Wilder*[58] *felt that this lesion if it can be called such* although more frequent in diabetic than in nondiabetic persons was probably a result rather than a cause of diabetes In its most striking form the islands



Fig 19 —Pancreas showing lipomatosis and moderate fatty infiltration of islet epithelium DA26-35 Female aged fifty four years Duration of diabetes five years *Sudan IV stain* × 310

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|---|---|---|---|---|---|---|---|---|---|
| Cecil[1] | 90 | 0 | 27 | 46 | 0 | 0 | 2 | 1 | 41 |
| Wilder[44]* | 29 | 8 | 4 | 17 | 0 | 0 | 0 | 5 | 0 |
| Cobb and Logan[22] | 142 | 11 | 30 | 79 | 0 | 15 | 7 | 0 | 0 |
| Herxheimer[43] | 97 | 22 | 38 | 11 | 0 | 0 | 0 | 0 | 0 |
| Warren and LeCompte | 811 | 271 | 333 | 186 | 32 | 20 | 16 | 11 | 63 |
| Total | 1169 | 312 | 432 | 339 | 32 | 35 | 25 | 17 | 104 |

* Only those of Wilder's cases are included in which the pancreas was specifically described.

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Enumerating the islands in the human pancreas is extremely difficult and for practical purposes only rough estimates are possible based on the total number of islands in a number of representative sections taken from different portions of the gland. Any method such as this, of course, is exposed to [illegible]

the islands in experimental animals are extremely valuable there but are rarely applicable to human tissues. Clark[45] working in Bensley's laboratory was able to count fairly accurately the number of islands in the pancreas of seven human adults. He found the number to range from 120 000 to 1 760 000. As noted in Chapter 2, Ogilvie found values in infants ranging from 117 226 to 2 325 123 and Bargmann[46] records counts in 5 adults varying from 208 309 to 1 760 000. (*See* Chapter 2 for Ogilvie's data on the weight of the normal island tissue.)

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to imperfect techniq... found diminished numbers in 13 cases ... Among Cecil's[4] 90 cases 20 showed few islands as did all of Weic... and Stangl's[64] 18 cases

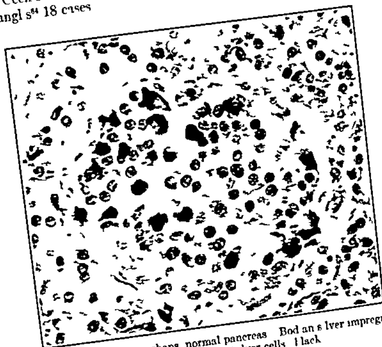


Fig 20 —Island of Langerhans normal pancreas Bodian silver impregnation after Bouin fixation Silver cells black

Kraus[65] reported that the number of islets per 50 cu mm of pancreas in 20 diabetics varied from 8 7 to 128 with an average of 54 1 This he stated gave a ratio of normal to diabetic of 2 4 to 1 Susman[66] using camera lucida drawings estimated the area of island tissue in proportion to pancreatic tissue in each of 10 microscopic fields in 202 cases including 51 diabetics Of 51 diabetics 29 had islet area readings below 0 9 per cent 14 were from 0 9 to 1 3 per cent 2 from 1 4 to 1 7 per cent 4 from 1 8 to 2 6 per cent 1 from 2 7 to 3 6 per cent and 1 was over 4 6 per cent He considers the range 0 9 to 2 7 per cent as normal for adults and 0 9 to 3 6 per cent as normal for infants Values over 3 6 per cent he thought were likely to be associated with hypoglycemia The results are of interest but one may question whether counting the islands in only 10 microscopic fields is sufficient to give an accurate picture of the mass of insular tissue in a given pancreas Ogilvie[48] warns that reduction of island tissue is still unproven as a factor of genuine etiological significance especially in view of the fact that experimentally one-eighth of the pancreas is enough to prevent diabetes

Congenital hypoplasia of the pancreas very rarely occurs (6 cases, 3 associated with diabetes mellitus)[47] and is apparently due to absence of the dorsal pancreatic anlage.

Moore[48] has reported a striking case of aplasia of the islands occurring in a thirteen-year-old girl whose diabetes had existed at least six years. Numerous sections of the pancreas revealed no normal islands, only a few small cell groups suggesting insular cells being present. Sections of the



Fig. 21. Two Islands of Langerhans in a diabetic pancreas. Frozen section. Gros-Schultze stain. Silver cells black. × 100

pituitary and hypothalamic regions were essentially negative. Dr. Lukens' case is described in Chapter 18.

Binger and Keith[49] reported an extraordinary case of fibrosis of the pancreas with generalized edema. No islands were found (special stains apparently were not used) and yet the patient was *not* diabetic.

As regards modification of the total mass or volume of the islands by various stimuli, the experimental results of Haist *et al.*[20] are of great interest. They used Bensley's method of vital staining of the islands with neutral red, then estimated the volume by pressing the pancreas between glass plates, projecting the islands on a screen, and tracing the area with a planimeter. From this they calculated the total weight of the insular tissue. They found that the weight of the islets increases with age in the rat. Factors reducing islet growth were (1) undernutrition, (2) insulin administration, and (3) hypophysectomy. Procedures stimulating islet growth were (1) continuous injection of glucose, (2) injection of crude extracts of pituitary gland, and (3) administration of thyroid extract.

The important critical work of Tejning[71] on methods for ascertaining the volume of the "islet organ" and the results of feeding various diets are described in Chapter 2.

It would seem desirable to combine some of these methods for calculating the total volume or mass of island tissue with the differential stains mentioned in the next section. Obviously it would be useful to know whether an increase or decrease of insular mass involved predominantly the α cells or the β cells.

**Differential Counts of Cells of Islands**.—It is only in recent years with the development of reasonably satisfactory staining techniques applicable to human post mortem material, that attempts have been made to do differential counts of the various cell types in diabetes. Foremost among the workers in this field is Gomori[7] who in 1941 described the chrome-alum hematoxylin and phloxine stain which is still perhaps the best available method for differentiation of α and β cells. In the same paper this author

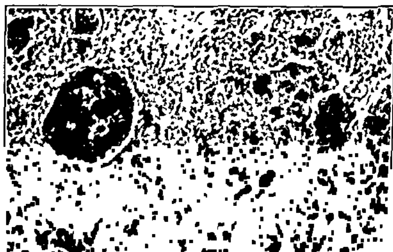


FIG 22.—Pancreas of 50-year old woman. Diabetes unrecognized before death. Cros-Schultze stain on frozen section showing two islets consisting almost entirely of α cells (black). × 150.

described differential counts done on several diabetic pancreases by his method. In some of these there appeared to be a definite reduction in the ratio of β to α cells but the results were considered of questionable significance.[73 74] More recently Gomori[75] writes that he has done differential counts on the islets in over 50 cases of diabetes and has obtained normal differential counts in 60 per cent. His most recent staining technique[76] (*see* Appendix) may prove of great value in this respect.

Bell[77] applied Gomori's chrome-alum hematoxylin method to 30 diabetic pancreases and found the β granules to be completely absent in 11 of these and present in very small quantity in 10 others. However 2 cases with normal granulation were instances of clinically severe diabetes. Although admitting that the significance of the β granules is still not clear

Bell[78] concluded that a histological diagnosis of diabetes could be made in this way in about 80 per cent of cases. Degranulation of the β cells is readily produced experimentally by hyperglycemia (Barron,[79] Peterson[80]) and hence it seems possible that it may have no more specific significance than this when encountered in the human pancreas, but may be useful in diagnosis in the same way that glycogen in the renal tubules is useful.

Hartroft[81] using phase contrast microscopy suggests that under certain conditions the size of the granules may provide a more sensitive index of the endocrine activity of the β cell than the number of granules.

Outstanding among the recent students of the subject is Ferner[82-84] who, as noted in Chapter 2, has applied the Gros-Schultze silver stain to the islands and on the basis of his findings has formulated a theory which holds that the "silver cells" (*including the α cells*) *are the precursors of the islands* ("inselpotente"). According to this point of view, the α cells represent immature ("unreifen") elements which are capable of differentiating into β cells under proper stimulation. According to Ferner this accounts for the apparent increase in islands with absence of symptoms in the newborn infant as compared with the adult (the infant's islands are predominantly α cells).

Ferner has applied the Gros-Schultze stain to the diabetic pancreas with interesting results. In only 3 of 17 diabetics was he unable to demonstrate an increase in "silver cells" (in addition to the α cells of the islands he includes cells scattered among the acini and in the ducts and states incidentally that the latter cells are not well demonstrated by other staining techniques). He seems to be convinced that the increase in α cells is an absolute, not a relative, one and interprets this phenomenon as indicating an attempt at regeneration, thus confirming his hypothesis that the α cells are precursors of the β cells. As additional evidence he finds buds of silver cells, especially in diabetic children, projecting from the ducts ("Inselprossen" and "Inselzapfen"). He describes in detail the case of a four year old diabetic child whose pancreas showed numerous islands and island buds almost completely composed of silver cells.

These observations led Ferner to formulate the hypothesis that diabetes, especially the juvenile type, is fundamentally due to a failure of silver cell (including the α cells) to ripen into β cells, i.e., he conceives of diabetes as essentially due to a *maturation arrest* of the cells of the islands. From analogy with the increase of silver cells which he was able to produce in the [illegible]

means of stimulating the silver cells to mature into insulin producing elements.

Ferner's concept, whether or not it is borne out by subsequent work, is provocative and worthy of careful consideration. One wonders if his maturation arrest, if indeed it exists, might not be analogous to similar situa-

tions affecting the bone marrow due to a deficiency (*e g* pernicious anemia) or to "toxic" factors (benzol other drugs) Certainly the inability to form new $\beta$ cells would seem to be the fundamental defect in the diabetic patient

As noted in Chapters 2 and 26 Ferner has recently modified his earlier views to some extent to take cognizance of the newer thoughts regarding possible production of the H G factor or glucagon by the $\alpha$ cells and has *indulged in interesting speculation on the possible role of the $\alpha$ cells* not only in normal carbohydrate metabolism but in cases of insulin resistance

Hess[87] seems to accept Ferner's conclusions without question In his own work he applied the Gros-Schultze method to 9 normal and 10 diabetic pancreases and computed the ratio of $\beta$ to $\alpha$ cells after counting 30 or more islets in each pancreas The mean ratio for the normals was 8 6 to 9 9 for the diabetics 2 1 to 4 8 The normals had a greater range of distribution in the counts while the diabetics were fairly closely grouped However remarkably enough there was no overlapping in the mean ratios the highest value for a diabetic was 4 8 the lowest normal 8 8 with none between these *Hess maintains that determination of the $\beta$ $\alpha$ cell ratio by* this method is the most reliable method for the histological diagnosis of diabetes from the pancreas and states that one need count only 20 to 30 islets in order to make the diagnosis This conclusion may be readily *criticized as based on too few cases A large number of pancreases should* be studied by his method in order to determine its value That it may have validity in some cases is suggested by the figures in Table 8 representing counts of 20 islands on frozen sections stained by *the Gros-Schultze method* on one normal and two diabetic pancreases *i e* an attempt to imitate Hess's technique as closely as possible

TABLE 8 DIFFERENTIAL COUNTS ON THREE PANCREASES

| | *Total cells in 20 islets* | | *Ratio* |
|---|---|---|---|
| | $\beta$ | $\alpha$ | $\beta$ $\alpha$ |
| A50-40 (normal) | 1362 | 161 | 8 4 |
| A50-38 (diabetic) | [illegible] | 214 | 4 [illegible] |
| U50 5 (diabetic) | 561 | 210 | 2 [illegible] |

*Variation in the size of the islands in the three cases is* of course indicated by the considerable differences in the total number of cells in 20 islands in each instance Obviously a great deal more work should be done along these lines

In what appears to be a careful piece of work Terbruggen[88, 89] tried to

Appendix A) He found
nondiabetics while in 80 per cent *of diabetics the ratio was less than* 3

*Table 9 is reproduced from Terbruggen* [illegible] *representing his counts on 64* nondiabetics and 26 diabetics of varying ages (but all over sixteen)

TABLE 9.—RANGE OF DIFFERENTIAL COUNTS IN 64 NONDIABETICS AND 26 DIABETICS (from Terbrüggen[illegible])

| | *Diminution of β cells* | | *Normal range* | | *Increase of β cells* | |
|---|---|---|---|---|---|---|
| Ratio of α to β cells | 1:1 to 1:2 | 1:2 to 1:3 | 1:3 to 1:4 | 1:4 to 1:5 | 1:5 to 1:6 | 1:6 to 1:7 |
| Nondiabetics | 0% | 23% | 29% | 29% | 15% | 4% |
| | 23% | | 58% | | 19% | |
| Diabetics | 38% | 42% | 12% | 8% | 0% | 0% |
| | 80% | | 20% | | 0% | |

It is obvious that there is some overlapping. However he holds that the ratio is never above 5 in a diabetic and in this respect agrees with Hess.[illegible] The question arises as to whether a reduced ratio of β to α cells represents (a) an absolute decrease in β cells or (b) a *relative* decrease in β cells, *i.e.* an increase in α cells. The latter possibility, as indicated by Lerner, must be considered particularly in juvenile diabetics. Terbruggen thought the islets were usually reduced in number in the diabetic and in many cases the weight of the pancreas as a whole seemed to be reduced. He therefore concluded that probably the whole island apparatus of the diabetic contains fewer β cells than that of nondiabetics. Terbruggen does not accept without reservation the hypothesis of Lerner that the α cells are precursors of the β cells; he points to the frequency of mitotic figures in β cells and also to studies of his own showing that in insular adenomas β cells may arise from nongranular precursors, thus indicating that α cells are not obligatory precursors of β cells. He suggests that the α cell may have a special function of its own.

We have tried the acid fuchsin and methyl green method as described by Terbruggen with some success. However, all the aniline dye techniques seem subject to slight variations in color values which at times make differential counts difficult or impossible. Herein lies the great advantage of the Gros-Schultze stain when properly carried out; the clarity of the picture facilitates counting, although it must be admitted that this advantage is partly counterbalanced by the thickness of the frozen sections, also by the capriciousness of the stain.

Gomori's[illegible] new aldehyde-fuchsin stain for the β cells shows great promise and may prove to be the most generally satisfactory method for differential counts yet devised.

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| | Total cells in 20 islets | | Ratio |
|---|---|---|---|
| | $\beta$ | $\alpha$ | $\beta$ $\alpha$ |
| A50-40 (normal) | 1362 | 163 | 8 4 |
| A50-38 (diabetic) | 99[illegible] | 214 | 4 [illegible] |
| [illegible]50-[illegible] (diabetic) | [illegible]64 | 210 | 2 [illegible] |

Variation in the size of the islands in the three cases is of course indicated by the considerable differences in the total number of cells in 20 islands in each instance Obviously a great deal more work should be done along these lines

In what appears to be a careful piece of work Terbruggen[88, 89] tried to confirm Ferner s results using not only the Gros-Schultze method but also a simple modification of Bensley s acid fuchsin methyl green method (see Appendix A) He found that the $\beta$ $\alpha$ ratio was between 3 and 5 for most nondiabetics while in 80 per cent of diabetics the ratio was less than 3

Table 9 is reproduced from Terbruggen[88] representing his counts on 64 nondiabetics and 26 diabetics of varying ages (but all over sixteen)

Table 9.—Range of Differential Counts in 64 Nondiabetics and 26 Diabetics (from Terbruggen[88])

| | *Diminution of β cells* | | *Normal range* | | *Increase of β cells* | |
|---|---|---|---|---|---|---|
| Ratio of α to β cells | 1 1 to 1 2 | 1 2 to 1 3 | 1 3 to 1 4 | 1 4 to 1 5 | 1 5 to 1 6 | 1 6 to 1 7 |
| Nondiabetics | 0% | 23% | 29% | 29% | 15% | 4% |
| | 23% | | 58% | | 19% | |
| Diabetics | 38% | 42% | 12% | 8% | 0% | 0% |
| | 80% | | 20% | | 0% | |

It is obvious that there is some overlapping However, he holds that the ratio is never above 5 in a diabetic and in this respect agrees with Hess[87] The question arises as to whether a reduced ratio of β to α cells represents (a) an absolute decrease in β cells or (b) a *relative* decrease in β cells i e an increase in α cells The latter possibility as indicated by Ferner must be considered particularly in juvenile diabetics Terbruggen thought the islets were usually reduced in number in the diabetic and in many cases the weight of the pancreas as a whole seemed to be reduced He therefore concluded that probably the whole island apparatus of the diabetic contains fewer β cells than that of nondiabetics Terbruggen does not accept without reservation the hypothesis of Ferner that the α cells are precursors of the β cells he points to the frequency of mitotic figures in β cells and also to studies of his own showing that in insular adenomas β cells may arise from nongranular precursors thus indicating that α cells are not obligatory precursors of β cells He suggests that the α cell may have a special function of its own

We have tried the acid fuchsin and methyl green method as described by Terbruggen with some success However all the aniline dye techniques seem subject to slight variations in color values which at times make differential counts difficult or impossible Herein lies the great advantage of the Gros-Schultze stain when properly carried out the clarity of the picture facilitates counting although it must be admitted that this advantage is partly counterbalanced by the thickness of the frozen sections also by the capriciousness of the stain

Gomori's[78] new aldehyde-fuchsin stain for the β cells shows great promise and may prove to be the most generally satisfactory method for differential counts yet devised

tions affecting the bone marrow, due to a deficiency (e.g., pernicious anemia) or to "toxic" factors (benzol, other drugs). Certainly the inability to form new β cells would seem to be the fundamental defect in the diabetic patient.

As noted in Chapters 2 and 26, Lerner has recently modified his earlier views to some extent to take cognizance of the newer thoughts regarding possible production of the "H G factor" or "glucagon" by the α cells and has indulged in interesting speculation on the possible role of the α cell not only in normal carbohydrate metabolism but in cases of insulin resistance.

Hess[87] seems to accept Lerner's conclusions without question. In his own work he applied the Gros-Schultze method to 9 normal and 10 diabetic pancreases and computed the ratio of β to α cells after counting 30 or more islets in each pancreas. The mean ratio for the normals was 8.6 to 9.9, for the diabetics 2.1 to 4.8. The normals had a greater range of distribution in the counts while the diabetics were fairly closely grouped. However, remarkably enough, there was no overlapping in the mean ratios: *the highest value for a diabetic was 4.8, the lowest normal 8.8, with* none between these. Hess maintains that determination of the β : α cell ratio by this method is the most reliable method for the histological diagnosis of diabetes from the pancreas and states that one need count only 20 to 30 islets in order to make the diagnosis. This conclusion may be readily criticized as based on too few cases. A large number of pancreases should be studied by his method in order to determine its value. That it may have validity in some cases is suggested by the figures in Table 8 representing counts of 20 islands on frozen sections stained by the Gros-Schultze method on one normal and two diabetic pancreases, i.e., an attempt to imitate Hess's technique as closely as possible.

TABLE 8 — DIFFERENTIAL COUNTS ON THREE PANCREASES

| | *Total cells in 20 islets* | | *Ratio* |
|---|---|---|---|
| | β | α | β : α |
| A50-40 (normal) | 1362 | 163 | 8.4 |
| A50-38 (diabetic) | 996 | 214 | 4 |
| A50-5 (diabetic) | 564 | 210 | 2 |

Variation in the size of the islands in the three cases is of course indicated by the considerable differences in the total number of cells in 20 islands in each instance. Obviously a great deal more work should be done along these lines.

In what appears to be a careful piece of work Terbruggen[88, 89] tried to confirm Lerner's results using not only the Gros-Schultze method but also a simple modification of Bensley's acid fuchsin methyl green method (see Appendix A). He found that the β : α ratio was between 3 and 5 for most nondiabetics, while in 80 per cent of diabetics the ratio was less than 3.

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TABLE 9.—RANGE OF DIFFERENTIAL COUNTS IN 64 NONDIABETICS AND 26 DIABETICS (from Terbruggen[88])

| | *Diminution of β cells* | | *Normal range* | | *Increase of β cells* | |
|---|---|---|---|---|---|---|
| Ratio of α to β cells | 1:1 to 1:2 | 1:2 to 1:3 | 1:3 to 1:4 | 1:4 to 1:5 | 1:5 to 1:6 | 1:6 to 1:7 |
| Nondiabetics | 0% | 23% | 29% | 29% | 15% | 4% |
| | 23% | | 58% | | 19% | |
| Diabetics | 38% | 42% | 12% | 8% | 0% | 0% |
| | 80% | | 20% | | 0% | |

It is obvious that there is some overlapping. However, he holds that the ratio is never above 5 in a diabetic and in this respect agrees with Hess.[87] The question arises as to whether a reduced ratio of β to α cells represents (a) an absolute decrease in β cells or (b) a *relative* decrease in β cells, *i.e.*, an increase in α cells. The latter possibility, as indicated by Lerner, must be considered particularly in juvenile diabetics. Terbruggen thought the islets were usually reduced in number in the diabetic and in many cases the weight of the pancreas as a whole seemed to be reduced. He therefore concluded that probably the whole island apparatus of the diabetic contains fewer β cells than that of nondiabetics. Terbruggen does not accept without reservation the hypothesis of Lerner that the α cells are precursors of the β cells; he points to the frequency of mitotic figures in β cells and also to studies of his own showing that in insular adenomas β cells may arise from nongranular precursors, thus indicating that α cells are not obligatory precursors of β cells. He suggests that the α cell may have a special function of its own.

We have tried the acid fuchsin and methyl green method as described by Terbruggen with some success. However, all the aniline dye techniques seem subject to slight variations in color values which at times make differential counts difficult or impossible. Herein lies the great advantage of the Gros-Schultze stain when properly carried out; the clarity of the picture facilitates counting, although it must be admitted that this advantage is partly counterbalanced by the thickness of the frozen sections and also by the capriciousness of the stain.

Gomori's[78] new aldehyde-fuchsin stain for the β cells shows great promise and may prove to be the most generally satisfactory method for differential counts yet devised.

Creutzfeldt [80] in a study of alloxan diabetes in the dog including many observations on the histo-physiology of diabetes in general remarks on the variation in the results of differential counts by different authors and notes the difficulty just mentioned of counting cells in thick frozen sections (Hess also mentioned an error of at least 10 per cent in his counts) Creutzfeldt found no evidence in the dog to support Ferner s thesis that $\alpha$ cells can be transformed into $\beta$ cells He observed no evidence of such transformation in the alloxan treated dog and thought that since the $\alpha$ cells persist apparently undamaged they must have a function He obtained a $\beta$ $\alpha$ cell ratio of 8 1 for the normal dog

To summarize the newer work on differential counts the varying figures for the *normal* $\beta$ $\alpha$ cell ratio obtained by various authors may be assembled Ferner 5 1 Hess 9 1 Terbruggen 4 1 Gomori 3 2 to 9 1 Gomori s recent statement[75] that 60 per cent of diabetics may have normal differential counts should also be mentioned again The differences are unexplained they may be due to staining techniques quantity or quality of material methods of counting (especially on frozen sections) or other unknown factors Certainly since most authors found some normals in the diabetic range and often some diabetics in the normal range it seems obvious that a post mortem diagnosis of diabetes cannot be made by this method with 100 per cent accuracy as Hess seems to suggest How often such a diagnosis can be made remains for further investigation to tell no doubt it is worth trying some of the methods mentioned in questionable cases since a very low $\beta$ $\alpha$ ratio is certainly almost conclusive evidence of diabetes (*see* Chapter 27)

Surprisingly little work has been done (probably in part because of the tedious methods required) on the total mass of island tissue both *absolute* and in relation to the weight of the pancreas It would seem desirable to apply techniques such as those of Ogilvie [45] Haist [78] and Su min[88] to the human diabetic pancreas in conjunction with the differential stains mentioned above in order to establish the presence or absence of a reduction in the *total mass* of $\beta$ cells (and perhaps increase in $\alpha$ cells) in human diabetes Conceivably the total volume of island tissue might be reduced to *such an* extent in some cases that a normal differential count might be associated [illegible] ( $\beta$ cell )

[illegible] a hyper [illegible]

more or less oppo es that of insulin it will be essential to estimate the quantities of both types of cells

It would be desirable also to correlate quantitative studies on the two types of cells in the islets with the recent work of Wrenshall *et al* [30a] on the extractable insulin of the pancreas These investigators found very

little insulin in the pancreases of severe diabetics with onset in youth ("growth-onset" type) and relatively larger amounts in subjects who had reached full stature prior to discovery of diabetes ("maturity-onset" type) This important work is considered further in Chapter 26

## CHANGES INVOLVING THE PANCREAS AS A WHOLE

Aside from the insular changes, changes in the pancreas as a whole must be considered particularly those involving the acinar cells

**Fibrosis** —Fibrosis is perhaps the simplest and most frequently occurring change Normally there is only a relatively delicate fibrous tissue septum

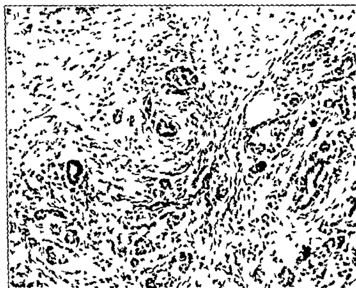


Fig 23 —Marked fibrosis of pancreas both interlobular and interacinar No 311 Male aged seventy years Duration of diabetes thirty nine years

between the lobules of the pancreas and there is a delicate basement membrane rarely more than about 1 micron thick between the acini and the islands Fibrosis both interlobular and interacinar is not infrequent It may or may not be accompanied by a moderate degree of round-cell infiltration either lymphocytes or large mononuclear cells This change may vary from irregular patches of fibrous tissue scattered through the pancreatic substance often tending to center about the ducts, to the almost complete disappearance of the acinar tissue with dilatation of the ducts characteristic of obstruction such as one sees in ligation experiments

Of the 405 cases that showed varying degrees of fibrosis 189 showed slight fibrosis the bulk of which was interacinar 141 showed moderate fibrosis, again the bulk of which was interacinar rather than interlobular, and 74 showed marked fibrosis, again predominantly of the interacinar type

There is no definite relationship between the occurrence of island lesions and interacinar fibrosis However, severe cases of either hyalinization or fibrosis of the islands usually show at least a moderate degree of interacinar fibrosis



Fig 24 —Extreme lipomatosis of pancreas Note white epithelial tissue imbedded in large mass of fat Fat has been stained with Sudan IV to provide better contrast No 1890 Female aged sixty five years Duration of diabetes eight months Natural size

Vartiainen[91] found gross fibrosis of the pancreas 30 times in a series of 166 diabetics and not at all in a control series of the same number

**Lipomatosis** —Fatty infiltration or lipomatosis of the pancreas as a whole has been considered as characteristic of diabetes In Figure 24 is shown the extreme degree of fatty infiltration which can occur in a pancreas Here hardly any epithelial tissue remains the organ being converted into little more than a mass of fat and fibrous tissue The amount of pancreatic fat is very closely related to that of the normal body fat and probably represents simply the degree of fat storage rather than being associated with the diabetic process in any peculiar way Certainly marked lipomatosis is common in obese patients in the absence of diabetes (*See* Chapter 4)

Two hundred forty-one or 30 per cent of the cases in our series showed varying degrees of lipomatosis ranging from a slight deposit of fat among the acini and along the interlobular septa to an extremely marked riddling

of the pancreas with large and small deposits of fat frequently occurring in single cells.

Incidentally, it hardly need be remarked that the weight of a pancreas such as that illustrated in Figure 24 is of no significance unless the fat is extracted.

**Arteriosclerosis.** Marked sclerosis of the pancreatic vessels occurred in 10 cases. Arteriosclerosis of the pancreas is not nearly so common as is arteriosclerosis of the spleen, which lies in the same vascular circuit. While the splenic artery may be extremely sclerosed, calcified and tortuous, there frequently is relatively little change in its branches which lead



Fig. 25.—Lipomatosis. DA27 93. Female aged sixty years. Duration of diabetes five years. Note large amount of perilobular fat and scattered fat cells throughout acinar tissue. Note also large number of hyaline islands. Phosphotungstic acid hematoxylin stain. × 22.5.

to the pancreas and there frequently is very little change in the pancreatico-duodenal artery. With only 7.4 per cent of the pancreases showing a marked degree of arteriosclerosis, arteriosclerosis as a factor in the production of diabetes, while it cannot be ruled out, is hardly of outstanding importance. Moschcowitz[27] seems to be practically alone in maintaining that arteriosclerosis (of the hyperplastic type) may be of importance in leading to hyalinization of islets and causing diabetes in persons in the older age



Fig 26 —Marked fibrosis and lipomatosis of pancreas with practical disappearance of acinar tissue Note marked dilatation of ducts and large number of islands present in the fibrous tissue Change secondary to carcinoma of the head of the pancreas U21-33 Female, aged sixty nine years Duration of diabetes eleven years Eosin methylene blue stain × 15

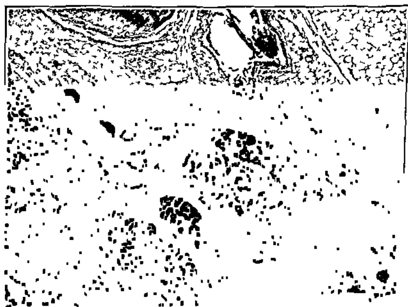


Fig 27 —Pancreas sh ... rosis and li ... te mucous glands in left lower corner ... lectic I ... e stain × 15

**Pancreatitis** —Acute pancreatitis appeared as a causal factor in 5 cases of this series Here we have the development of diabetes simply through destruction of a considerable proportion of the pancreatic tissue just as one might surgically remove a sufficient amount of the pancreas to cause



Fig 28 —Longitudinal section of pancreas from case of acute hemorrhagic pancreatitis Note white fat necrosis No 537 Male aged seventy-one years Nondiabetic



Fig 29 Section through head of pancreas from same case as Figure 28 showing marked necrosis and hemorrhage Natural size

the development of diabetes experimentally The excessively rare authentic cases of post traumatic diabetes such as Wells' case [93] probably rest on destruction of insular tissue in the course of pancreatitis due to the trauma

Probably cases of diabetes after acute pancreatitis would be more frequent were it not that the lesions which destroy enough of the pancreas to

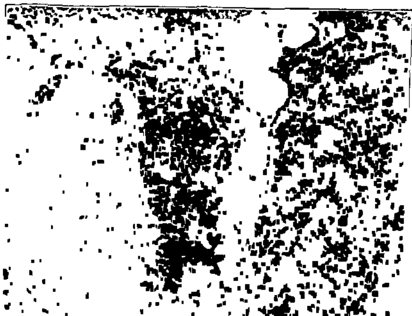


Fig 30 —Acute pancreatitis showing necrosis of pancreatic tissue, fat necrosis fibrin deposits and leukocytic infiltration No 537 Male aged seventy-one years Non diabetic Iron hematoxylin Van Gieson stain × 150



Fig 31 —Acute pancreatitis periductal Note polymorphonuclear leukocytes within ducts and infiltration of wall A14 79 Eosin methylene blue stain × 110

produce insulin deficiency are generally sufficiently severe to cause death from the pancreatitis itself, before there has been opportunity for diabetes to demonstrate itself.

A most interesting series was presented by Bernhard,[94] who studied 50 cases of operative recovery from acute pancreatitis. Five developed diabetes, 3 dying of the disease and 2 living with severe diabetes. The time interval between pancreatitis and diabetes ranged from immediately subsequent to thirteen years.

Gambill *et al.*[95] report a case of diabetes associated with acute hemorrhagic pancreatitis.

Increasing awareness of the possible relation between chronic pancreatitis and diabetes is evident in the recent literature. Comfort *et al.*[96] found diabetes in 7 of their 29 cases of chronic relapsing pancreatitis. Maimon *et al.*[97] obtained a diabetic type of glucose tolerance curve in 8 of 9 cases of chronic pancreatitis, suggesting that this test might uncover many more cases if used more often. Little attention seems to have been paid to the possibility that mumps may account for a large percentage of cases of chronic pancreatitis.[98, 99, 100] Shumacker[101] and Paxton and Payne[102] call attention to alterations in glucose tolerance in acute pancreatitis.

Also, as emphasized by Root,[103] it is probable that more consideration should be given to acute pancreatitis as a precipitating cause of coma in a recognized diabetic.

This series includes in addition to the 5 cases where pancreatitis was apparently the cause of their diabetes 12 cases of pancreatitis developing six months to thirteen years after the onset of diabetes. Two showed pancreatic apoplexy, four showed acute pancreatitis and six subacute pancreatitis.

It must be true of course that some of the cases of fibrosis mentioned above represent healed pancreatitis, but there would seem to be no means of determining the incidence other than by exhaustive clinical investigation.

**Abscesses.**—Four cases of metastatic abscesses in the pancreas were encountered, all in persons suffering from staphylococcus pyemia. No case has been encountered since the last edition.

**Syphilis.**—Pancreatic changes secondary to syphilis may cause diabetes but only rarely. Among 300 diabetic autopsies reported by Simmonds,[104] only 3 cases were found where the small fibrotic pancreas damaged by syphilis had led to diabetes. In neither of his 2 cases of gumma of the pancreas was glycosuria noted. A case of syphilis studied by Carnot and Harvier[105] died with severe diabetes and showed at autopsy syphilitic pancreatitis and gumma. Lemann[106] found but few cases of coexisting syphilis and diabetes.

**Calculi.**—In our early series of 327 autopsied diabetics pancreatic calculi were encountered three times. Wilder[35] reported 3 cases among 55 autopsied cases. One of his cases showed hemochromatosis also, another gall

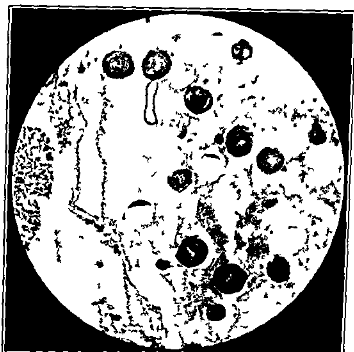


Fig 32 —Pancreatic cysts ith concret ons A17-6? Nond abet c × 22 5

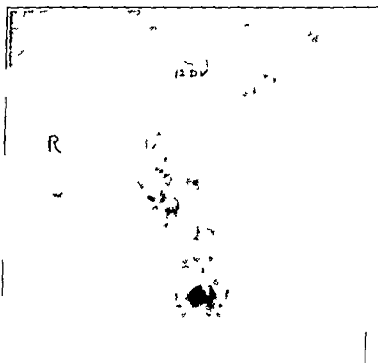



Fig 33 —Roentgenogram showing pancreat c calci ... d twenty three years
Duration of d a ... years ... 7)

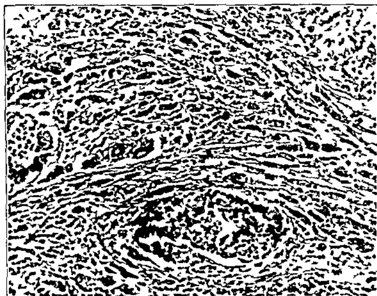


Fig 34.—Carcinoma of pancreas showing persistence of islands in the midst of tumor tissue No 1638 Male aged sixty-eight years Nondiabetic Phosphotungstic acid hematoxylin stain × 190



Fig 35.—Carcinoma of island-cell type simulating appearance of islands of Langerhans. Eosin methylene blue stain. × 250 (Drawing)

stones and the third cholecystitis Morrison and Bogan[107] have pointed out that the diagnosis can be made during life by roentgenogram Figure 36 taken from one of their plates shows multiple calculi in a diabetic girl aged twenty three years They reported 8 cases of which 4 were diabetic

Fischer[108] in a review of the literature stated that about half of 2?0 reported cases of pancreatic lithiasis had diabetes Edmondson *et al*[109] reported 30 per cent of 26 cases as having diabetes

The presence of calculi naturally predisposes to fibrosis of the pancreatic tissue, either through direct obstruction or tendency to localized infections with subsequent scarring In obstruction the effect is closely akin to that of duct ligation resulting in atrophy of the acinar tissue and persistence at least for a considerable time of the islands This lesion is of much historical interest as one case reported by Barron[110] aroused Banting's interest and thus played a part in the development of insulin

Diffuse calcification of the pancreas without calculi is apparently an exceedingly rare condition Nuzum[111] found 17 cases in the literature with evidence of disturbed carbohydrate metabolism in 45 per cent Present and Geyman[112] review the radiological findings

**Amyloidosis** —Since reactions characteristic of amyloid were not infrequently obtained in cases of hyalinization of the islands much attention was paid to the occurrence of amyloidosis *i e* involvement of the pancreas as part of a more or less general amyloid deposition in the usual organs (so-called typical amyloidosis) This was found in only 2 cases One a female aged sixty two years died of carcinoma of the uterus with diabetes of two years duration Calculi were also present in the pancreas The second a female aged fifty four years whose diabetes had been present for 4 9 years died of multiple abscesses and staphylococcus pyemia

For a discussion of the relation of atypical amyloid to hyalinization of the islets *see* page 33

**Carcinoma** While carcinoma is of relatively infrequent occurrence among diabetics (672 fatal cases among 8 384 diabetic deaths reported by Joslin[113]) carcinoma of the pancreas is not a rare lesion compared with other types of carcinoma Whereas the average incidence of carcinoma of the pancreas is only 4 per cent of all malignant tumors it was found by McKittrick and Root[114] in 32 4 per cent of the 37 cases of malignant disease in diabetics seen at the New England Deaconess Hospital during three and half years

Urmy *et al*[115] have reported a case of mild diabetes in a male aged forty six years developing two and a half years subsequent to the appearance of a slowly growing carcinoma of the pancreas In the 2 cases of Burkhardt[116] with carcinoma of the head of the pancreas obstructing the ducts clinical diabetes occurred in spite of the presence of regenerating island Benson and Gordon[117] found diabetes in 6 of 28 patients with cystadenoma of the pancreas

For a consideration of cancer in diabetes and especially carcinoma of the pancreas as an etiological factor see Chapter 24.

**Metaplasia of Duct Epithelium.**—This change, ranging from a mere heaping up of clusters of basal epithelial cells to complete keratinization, is not related to diabetes. Seen at its best in certain cases of A avitaminosis and "fibrocystic disease," it may be associated with retained secretion, calculi or other duct abnormalities. In this series 19 instances were encountered among 811 pancreases examined microscopically without significant age or sex distribution, the youngest being a woman of fifty-nine. Korpassy[115] found this change in 58 of 500 human pancreases.

**Quantitative Variations.**—So far as the size of the pancreas itself is concerned, there is relatively little indication one way or another as to the role that this plays. Herxheimer[59] noted the pancreas to be small, thin or heavily infiltrated with fat in 105 of 162 cases of diabetes mellitus. Of the 730 cases of this series in which the weight of the pancreas was known, there were 122 cases in which the pancreas was noted as definitely small or less than 50 grams, 481 cases in which the pancreas was normal or between 50 and 100 grams, and 127 cases in which the pancreas was large, over 100 grams. Of course the weight must be accepted with more than a grain of salt as the degree of fatty infiltration can only be estimated, and a pancreas, for example, may weigh as much as 240 grams and yet contain less pancreatic tissue than one weighing perhaps 50 grams.

In a group of 31 pancreases from diabetic patients Terplan[113] found 8 to be fatty. The weights ranged from 21 grams in a child fifteen years of age and 22 grams in an adult of fifty-two years to 110 grams.

Vartiainen,[91] in a careful gross study of the organs of 100 diabetics matched with controls of comparable age and sex, found that 22 of the diabetics had pancreases weighing less than 40 gm., while only 2 controls had pancreases of less than 40 gm.

Terbruggen[89] encountered small pancreases (17 to 40 gm.) quite frequently in his juvenile diabetic group.

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Chapter 4

# LESIONS OF THE PANCREAS IN NONDIABETIC INDIVIDUALS

To serve as a control for the lesions of the pancreas in cases of diabetes discussed in the last chapter the pancreas was studied in 200 consecutive autopsies performed on nondiabetic individuals in this laboratory in 1928 and a portion of 1929 The findings in the first 156 of these have been reported [1] The primary cause of death ranged from such chronic conditions as carcinoma multiple sclerosis and endocarditis to pneumonia meningitis and postoperative pulmonary embolus These cases are fairly representative of various ages ranging from one day to eighty seven years at the time of death However as may be seen from the tables they are rather weighted in the later decades Thus the age distribution is not unlike that of diabetic deaths In only 6 cases were pancreatic changes an important factor in the clinical course or the causation of death

So much attention has been paid to even minor lesions in the pancreas of diabetics without much regard to changes seen in the pancreas of nondiabetics that a tendency has developed to relate complacently almost all types of pancreatic lesions with diabetes

**Methods** — Sections of the pancreas were fixed routinely in Zenker's fluid and stained with phloxine-methylene blue and phosphotungstic acid hematoxylin In all autopsies performed under three hours post mortem a considerable number of the cases Zenker's Helly's and Champy's fluid were used for fixation as were formalin and Regaud's method For stains in addition to phloxine methylene blue and phosphotungstic acid hematoxylin the granule stains of Bensley Lane and Ukai Mallory's aniline blue and Masson's three-color stains were resorted to Serial sections were studied in some instances

In Table 10 are seen the lesions affecting the pancreas as a whole arranged by decades

In making the diagnosis of fibrosis only those cases showing clear-cut (though at times focal) increase in interacinar fibrous tissue with or without fibrous tissue increase in the interlobular septa were accepted and those cases which showed only slight fibrosis were omitted In a number of instances this fibrosis was accompanied by infiltration to varying degrees with large mononuclears lymphocytes and rarely eosinophiles In some instances entire lobules had disappeared with fibrous tissue and scattered islands and ducts remaining The tendency of this lesion to increase in the older age group is strikingly shown being entirely absent in the 11

cases under thirty, yet occurring in 36.6 per cent of the 157 cases over thirty years of age. While this frequency is markedly lower than in diabetic individuals in the same age groups, it is nevertheless so great as to preclude considering it a lesion characteristic of diabetes. In nondiabetic individuals it occurs nearly as frequently in association with normal islands as in those whose islands showed pathological change.

TABLE 10.—LESIONS OF THE ENTIRE PANCREAS IN NONDIABETICS

| Age, years | Cases | Fibrosis | Lipomatosis | Acute pancreatitis | Carcinoma | Metastatic tumors | Cysts | Calculi | Tuberculosis |
|---|---|---|---|---|---|---|---|---|---|
| 0 to 10 | 6 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 11 to 20 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 21 to 30 | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 31 to 40 | 10 | 3 | 0 | 0 | 0 | 0 | 0 | 1 | 0 |
| 41 to 50 | 34 | 3 | 5 | 2 | 0 | 1 | 0 | 0 | 0 |
| 51 to 60 | 63 | 23 | 10 | 0 | 1 | 6 | 1 | 0 | 0 |
| 61 to 70 | 52 | 17 | 8 | 2 | 0 | 6 | 0 | 0 | 1 |
| 71 to 80 | 28 | 10 | 4 | 1 | 0 | 1 | 1 | 0 | 0 |
| 81+ | 2 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Total | 200 | 58 | 27 | 5 | 1 | 14 | 2 | 1 | 1 |
| Per cent of total | | 29.0 | 13.5 | 2.5 | 0.5 | 7.0 | 1.0 | 0.5 | 0.5 |

It seems unlikely that interacinar fibrosis is a cause of atrophy of the pancreas. Probably the fibrosis is far more frequently a result of atrophy of the parenchymatous tissue from one cause or another with resultant condensation of the stroma than an active factor in the production of pancreatic damage. The analogy to the cirrhotic process in the liver is rather striking.

In many of the cases showing fibrosis there is evidence from the anatomical findings or from the history of previous gall bladder disease. In all of these cases of fibrosis fibrous-tissue capsules can be seen about the great majority of the islands, a finding which Otani considers definite evidence of a pathological process.

The criteria used for the diagnosis of lipomatosis are: (1) separation of the lobules by fat cells and (2) frequent fat cells occurring among the acini. In this series the presence of lipomatosis in the pancreas was closely related to the amount of body fat. Undoubtedly the proportion of cases showing this would have been much higher were it not for the varying degrees of emaciation, at times extending to almost complete depletion of the stored fat of the body, in the cases of malignant disease which were fairly frequent in this series of post mortem examinations. As with fibrosis, lipomatosis is associated with normal islands fully as frequently as with diseased islands.

Balo[3] found the dry weight of the pancreas after extraction of fat to be 11 to 20 grams in the normal pancreas and 5 to 12 grams in the fatty gland

It is rather difficult to determine whether stored fat is the only factor in producing this change or whether there is some deposit of fat in response to varying degrees of atrophy of the pancreatic parenchyma

Primary carcinoma occurred only once an adenocarcinoma of the head of the pancreas arising from the duct Many of the islands persisted apparently uninjured in the midst of the tumor tissue and those islands in the uninvaded portion of the pancreas were also normal They were not hypertrophic This incidence of 0 5 per cent contrasts with that of 2 per cent found in our diabetic autopsy series

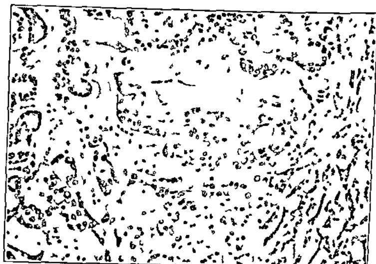


Fig 36 Carcinoma of head of pancreas duct type Metastasis to liver Hematoxylin eosin stain × 215

Metastatic tumors were frequently encountered as would be expected from the frequency of malignant disease in the post mortem material There were 14 cases of metastatic tumor or 7 per cent Of these 1 was malignant melanoma 1 myelogenous leukemia 1 lymphatic leukemia and the remainder carcinoma In several cases the degree of infiltration was striking but a sufficient number of islands persisted to maintain normal carbohydrate metabolism

Tuberculosis of the pancreas was encountered once as were calculi in the pancreatic ducts Cysts of the pancreas occurred twice once apparently the result of a localized acute pancreatitis with subsequent autolysis and once the result of obstruction to the duct of Wirsung by fibrosis of undetermined origin

Acute pancreatitis either hemorrhagic or gangrenous was encountered 5 times In four cases it was the primary cause of death and in 1 it was associated with a staphylococcus pyemia

**Lesions of the Islands.**—The occasional occurrence of lesions in the islands of nondiabetic individuals similar to those found in diabetes is a prerequisite if we consider insular changes the cause rather than the result of diabetes.

Table 11 gives the frequency of lesions in the islands.

TABLE 11.—LESIONS OF THE ISLANDS IN 200 NONDIABETIC PANCREASES

| Age, Yrs. | Cases | Normal | Fibrosis: Slight | Fibrosis: Moderate | Fibrosis: Marked | Hyaline: Slight | Hyaline: Moderate | Hyaline: Marked | Hypertrophy | Mitoses | Pyknotic nuclei | Hydropic change | Adenoma | Hemorrhage |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 0 to 10 | 6 | 6 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 11 to 20 | 2 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 21 to 30 | 3 | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 31 to 40 | 10 | 9 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 41 to 50 | 34 | 31 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 1 | 0 |
| 51 to 60 | 67 | 54 | 3 | 0 | 0 | 0 | 0 | 1 | 1 | 2 | 1 | 0 | 0 | 1 |
| 61 to 70 | 52 | 40 | 5 | 2 | 1 | 0 | 1 | 0 | 1 | 1 | 0 | 1 | 0 | 0 |
| 71 to 80 | 23 | 20 | 2 | 1 | 0 | 0 | 1 | 0 | 3 | 0 | 0 | 1 | 0 | 0 |
| 81+ | 2 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Total | 200 | 167 | 10 | 4 | 1 | 0 | 3 | 1 | 5 | 3 | 2 | 2 | 1 | 1 |

This table shows that practically all of the lesions of the islands that are described in diabetic individuals are to be found, although with less frequency, in nondiabetic individuals. Thus the islands were normal in 83.5 per cent of the cases. Fibrosis of the islands ranging from slight to moderate was present in 7.5 per cent, hyalinization of the islands was present in 2 per cent, hypertrophy of the islands in 2.5 per cent, mitotic figures in the island epithelium in 1.5 per cent, hydropic change (?) in 1 per cent, shrunken cells with pyknotic nuclei in 1 per cent, and hemorrhage in the islands and an adenoma of island type each in 0.5 per cent.

Presumably fibrosis of the islands may be brought about by the same causes that produce the interacinar fibrosis. This latter lesion appeared in 10 out of 15 pancreases that showed fibrosis of the islands. This is not quite so high a proportion as was noted in our first series.

Hyalinization of the islands has been considered perhaps the most characteristic lesion of diabetes. That the occurrence of this hyalinization was not safe ground for establishing a diagnosis of diabetes pathologically was shown by the case of Ohlmacher,[4] who reported the occurrence of hyaline islands in the pancreas of a nondiabetic, aged seventy-two years, under observation for nine months previous to death. Four-fifths of the islands showed hyaline degeneration, but many of the surviving ones were hypertrophied, which probably accounts for the absence of diabetic symptoms. Saltykow[5] reported hyaline degeneration of some of the islands of Langerhans in the pancreas of a male, aged eighty-eight years, with advanced arteriosclerosis. Cecil,[6] in a careful study of these lesions in nondiabetics

It is rather difficult to determine whether stored fat is the only factor in producing this change or whether there is some deposit of fat in response to varying degrees of atrophy of the pancreatic parenchyma

Primary carcinoma occurred only once an adenocarcinoma of the head of the pancreas arising from the duct Many of the islands persisted apparently uninjured in the midst of the tumor tissue and those islands in the uninvaded portion of the pancreas were also normal They were not hypertrophic This incidence of 0 5 per cent contrasts with that of 2 per cent found in our diabetic autopsy series

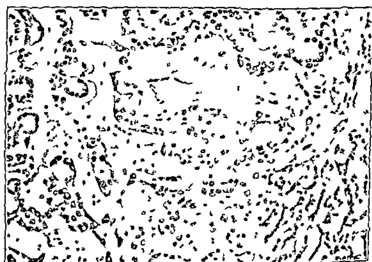


Fig 36 —Carcinoma of head of pancreas duct type Metastases to liver Hematoxylin eosin stain × 215

Metastatic tumors were frequently encountered as would be expected from the frequency of malignant disease in the post mortem material There were 14 cases of metastatic tumor or 7 per cent Of these 1 was malignant melanoma 1 myelogenous leukemia 1 lymphatic leukemia and the remainder carcinoma In several cases the degree of *infiltration* was striking but a sufficient number of islands persisted to maintain normal carbohydrate metabolism

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|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Slight | Moderate | Marked | Slight | Moderate | Marked | | | | | | |
| 0 to 10 | 6 | 6 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 11 to 20 | 2 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 21 to 30 | 3 | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 31 to 40 | 10 | 9 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 41 to 50 | 34 | 31 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 1 | 0 |
| 51 to 60 | 63 | 54 | 3 | 0 | 0 | 0 | 0 | 1 | 1 | 2 | 1 | 0 | 0 | 1 |
| 61 to 70 | 52 | 40 | 5 | 2 | 1 | 0 | 1 | 0 | 1 | 1 | 0 | 1 | 0 | 0 |
| 71 to 80 | 28 | 20 | 2 | 1 | 0 | 0 | 1 | 0 | 3 | 0 | 0 | 1 | 0 | 0 |
| 81 + | 2 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Total | 200 | 167 | 10 | 4 | 1 | 0 | 3 | 1 | 5 | 3 | 2 | 2 | 1 | 1 |

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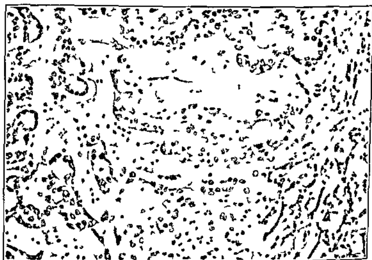


Fig 36 —Carcinoma of head of pancreas duct type Metastasis to liver Hematoxylin-eosin stain × 215

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| 0 to 10 | 6 | 6 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 11 to 20 | 2 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 21 to 30 | 3 | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 31 to 40 | 10 | 9 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
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| 51 to 60 | 63 | 54 | 3 | 0 | 0 | 0 | 0 | 1 | 1 | 2 | 1 | 0 | 0 | 1 |
| 61 to 70 | 57 | 40 | 5 | 2 | 1 | 0 | 1 | 0 | 1 | 1 | 0 | 1 | 0 | 0 |
| 71 to 80 | 28 | 20 | 2 | 1 | 0 | 0 | 1 | 0 | 3 | 0 | 0 | 1 | 0 | 0 |
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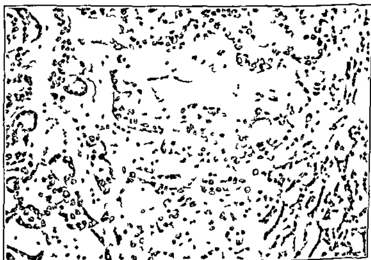


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uals is similar to that occurring in the islands of diabetic individuals Stains for glycogen were not used

Again hemorrhage in the island is probably an artifact or at least an agonal change This lesion was encountered once in the present series

The pancreatic tissue is fairly insusceptible so far as actual necrosis is concerned to injury from toxic causes as compared with such tissues as liver or kidney and so regenerative changes are rarely seen This probably accounts for the rarity of diabetes after infections Mitosis are occasionally encountered in the island cells (3 times in this series) but no more frequently than would be expected in slow replacement of worn-out cells

The curious epidermoidalization of the ducts to which attention has been called by Balo and Ballon [16] Priesel [17] Yotuyanagi [18] and others was found only once in this series

**Summary** —It is striking that practically any lesion found in the pancreas of diabetic individuals either involving the island tissue the acinar tissue or both can be duplicated in the pancreas of nondiabetics However the frequency of lesions of the islands is much less in nondiabetic individuals than in diabetics Therefore the occurrence of any of the generally accepted insular lesions may be considered as presumptive evidence of diabetes However without studies of the glycogen distribution in other organs and perhaps differential counts on the islands as well the diagnosis of diabetes cannot be made Interstitial pancreatitis as represented by fibrosis occurs too frequently and in too wide a range of disease processes to be considered a characteristic lesion of diabetes Lipomatosis is probably more nearly related to the amount of stored fat in the body than it is to pathological changes in the pancreas

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reports 5 cases of his own and one of Whipple s In addition he states that Milne and Peters[7] mentioned hyaline degeneration of the islands in non diabetic conditions

Wright[8] has reported 5 cases all over fifty years of age showing varying degrees of hyalinization of the islands associated with slight to marked interacinar fibrosis and showing slight to moderate arteriosclerosis In this series 3 cases of moderate hyalinization of the islands were encountered and one of marked hyalinization all over forty years of age The youngest case one of Cecil s was thirty seven years of age Arrangement and staining properties of the hyaline in nondiabetics are identical with those of the islands of diabetic individuals

The staining reactions of amyloid may also be found in the hyaline of nondiabetic islands Arey[9] found hyaline which he regarded as amyloid in 16 6 per cent of his nondiabetic series and Ahronheim[10] obtained positive reactions for amyloid in 5 of 50 nondiabetics

Lymphocytic infiltration of the islands was found by Susman [1] in a nondiabetic woman aged forty six years who died of Addison s disease secondary to adrenal atrophy

*Hypertrophy of the islands was encountered in 5 cases and 4 of these* showed fairly marked interstitial pancreatitis There is some discrepancy in statements as to the normal size of the islands However a diameter of 400 micra has been considered the upper normal limit by Cecil [1] and this has been used as the criterion in the present study

There is one difference however between the hypertrophied islands of the diabetic and the nondiabetic Frequently the hypertrophied islands *of a diabetic individual consist of elongate cells* with narrow centrally located nuclei whereas the hypertrophied islands in the pancreas of nondiabetic individuals are made up of the usual polyhedral cells with rounded or slightly oval nuclei

In relation to the work of Ferner cited in Chapter *one wonder* whether this difference may not be due at least in some cases to a predominance of $\alpha$ cells in the hypertrophied island of the diabetic

Adenoma of island-cell type is a rare lesion In 1926 one of us (S W) reported [3] 4 cases and collected 16 others from the literature In only one case that of Heiberg [14] was diabetes present In the present series one case was found occurring in a female aged forty four years dying from *epidermoid carcinoma of the cervix uteri Further* discussion of insular adenomas may be found in Chapter 22

It is probable that the occurrence of pyknotic nuclei in the island cells is an artifact at least in nondiabetics but as this has been considered as a possible diabetic lesion (Gibb and Logan[15]) *the occurrence has been* recorded in Table 11

Similarly hydropic change at least so far as it occurs in nondiabetics is probably due to post mortem change However its appearance in the 2 instances in which it occurred in the present series of nondiabetic individ

Chernick *et al.*[illegible] showed that the conversion of $C^{14}$-labeled glucose to fatty acids is drastically reduced in liver slices of alloxan-diabetic rats confirming Stetten's conclusion that the failure to utilize glucose for fatty acid synthesis is a major metabolic defect in diabetes. They also found that insulin would completely repair this defect.

It has also been shown[8] that the liver is the site of the conversion of glucose to long-chain fatty acids (as well as short chain) and that in diabetic rats these same fatty acids are readily converted back to glucose. Hastings and his co-workers[9-10] have studied various aspects of the synthesis of glycogen by liver slices *in vitro* including the incorporation of $CO_2$, derivation of glycogen from acetate and pyruvate, effects of the ionic environment, etc.

Chaikoff[illegible] has summarized some of the newer contributions in this field.

## GLYCOGEN DISTRIBUTION

Although diabetes mellitus is primarily a disease of carbohydrate metabolism we know very little from the histological standpoint regarding it. The solubility of the various sugars in the body fluids makes it practically impossible to utilize any of the ordinary histological procedures in study of the problem. The carbohydrate that we can study most readily by histological means, glycogen, is fortunately of great importance, and there are abnormalities of glycogen distribution that characterize diabetes. Some of these changes, such as the glycogenic infiltration of the renal tubular epithelium, were recognized long before their nature was understood. Thus Armanni described a hyaline vacuolization of the epithelium of Henle's loops unaware that it was due to the presence of glycogen within the epithelial cells.

Since Ehrlich in 188[illegible], found in 14 cases of diabetes this peculiar infiltration of the epithelium of Henle's loops in the kidneys to be due to the presence of glycogen, this lesion has been regarded as the most definite anatomical finding in active diabetes mellitus. Other less constant abnormal glycogenic deposits associated with the disease were also noted by Ehrlich: the increased glycogen in the heart muscle fibers, glycogenic granules in the leukocytes and large glycogen-filled vacuoles in the liver cells. Curiously enough it was not until twenty-three years later that Meixner seen followed by Best demonstrated the glycogen-filled vacuoles in the epithelial cells of the liver to be the nuclei.

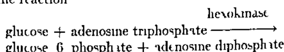
**Methods.** While Best's carmine stain is still a useful method, the best available technique appears to be the periodic acid-Schiff reagent method following fixation in chilled Rossman's fluid. Absolute fixation is, however, not essential, good results being obtainable after formalin (see Appendix 1).

In renal epithelium and liver-cell nuclei the presence of vacuolization as shown in paraffin sections with various stains is a further aid. This vacuolization runs closely parallel with the glycogen content as shown by Best's

# Chapter 5

# PATHOLOGICAL EVIDENCES OF ABNORMAL CARBOHYDRATE METABOLISM

RECENT advances in the physiology of the metabolism of carbohydrates have greatly exceeded those in the histological distribution and histochemistry of these substances. Stetten[1] has recently summarized modern concepts of the metabolism of carbohydrates. He emphasizes among other points that one may no longer speak of simple "oxidation" or "burning" of carbohydrate and that the boundary lines between the metabolisms of protein, fat and carbohydrate are no longer distinct since it has been shown that their catabolic pathways cross at many points and that each of these three major nutrients may supply organic fragments which feed into the tricarboxylic acid cycle. He also indicates the fundamental importance of the reaction



glucose + adenosine triphosphate —hexokinase→ glucose 6 phosphate + adenosine diphosphate

especially in view of the fact that the anterior pituitary seems to produce a specific inhibitor of the ubiquitous enzyme hexokinase (although it seems to affect the hexokinase of muscle and liver more than that of other cell types) and that insulin seems to act to antagonize or neutralize this inhibitor.

Much light is being shed on problems of carbohydrate and fat metabolism by the use of radioactive carbon ($C^{14}$). Thus Stetten[1] showed that of 30 gm. of carbohydrate given to the normal rat only 1 gm. is stored as glycogen, 10 gm. are stored as fat and 19 gm. are broken down into the "metabolic pool" for energy and for conversion to protein, fat or other substances. Zilversmit *et al.*[2] found that the over all rate of oxidation of glucose by the alloxan-diabetic rat did not differ significantly from that of the normal. Villee and Hastings[3] using the isolated rat diaphragm technique showed that when no insulin was added, glucose utilization and glucose metabolized to carbon dioxide were lower than normal in muscle from alloxan-diabetic rats and greater than normal in muscle from adrenalectomized, hypophysectomized, and adrenalectomized-hypophysectomized animals; also that glucose synthesis without insulin was lower than normal in diabetic and adrenalectomized rats and greater in hypophysectomized diabetic adrenalectomized and hypophysectomized adrenalectomized rats. The addition of insulin increased the amount of glucose appearing as $CO_2$ in all but the diabetic diaphragm, in which the increase was not significant.

heart, liver, kidneys, pancreas, voluntary muscle and skin. Both insulin treated cases of diabetes and cases not treated with insulin are included as well as a control series of nondiabetics.

*Heart.*—Glycogenic infiltration of the heart muscle is marked in many cases of diabetes. It is particularly striking in those fibers at the margins of infarcts. It can also be found there longer post mortem. Whether this means greater storage of glycogen or that the fibers are damaged and so deficient in glycogenolytic ferment is somewhat difficult to decide. On the basis of tissue fixed under thirty minutes post mortem an abnormally large storage of glycogen seems the more likely.

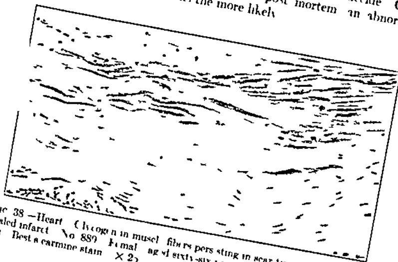


Fig 38.—Heart. Glycogen in muscle fibers persisting in scar tissue at the margin of a healed infarct. No 889. Female aged sixty-six years. Duration of diabetes fifteen years. Best's carmine stain. × 25.

So far as the amount of glycogen in the muscle as a whole is concerned there is but little difference between the insulin treated cases of diabetes and the control series. The diabetic cases not treated with insulin show a high proportion of heavy deposits of glycogen.

Cruickshank[10] has made an extensive study of cardiac glycogen showing that it is definitely and char ... ally increased in both human and experimental d ... ho ...
are not clear ... reasons for its deposition in this site
diabetic heart ... us and unexplained fact that the
normal, a ... to ... cent greater than the
and diabetic ... g 100 g for normal
has lost its p ... ests that the heart
... ut not the power
the presence o ... that in diabetes
... lso says that in
... of glycogen in

stain or the periodic acid method in tissues fixed under one hour post mortem, and is much more marked than the stain would indicate in tissues fixed a longer time post mortem

In most tissues there is a rapid drop in demonstrable glycogen in the first hour, and but little change for some hours thereafter Yater, Osterberg, and Hefke[16] found by microchemical analysis that one-half to three-fourths of the glycogen in the rabbit disappears in the first hour and that from then on the rate of decrease is slow



Once fixation has occurred, change in glycogen is slight or none But little change is detected in staining property of glycogen in absolute alcohol-fixed tissue after ten years Brian[17] found 3 per cent glycogen present in a liver Kaiserling-preserved for eighteen months Ordinary formalin will inhibit enzyme action and so prevent glycolysis Traces of both intracellular and intranuclear glycogen have been demonstrated in this laboratory in formalin-fixed diabetic livers after preservation for over twelve years

Old paraffin-embedded tissue is quite suitable for glycogen stains, even though the original fixative may have been in aqueous solution[18]

**Results.**—Both surgical and post-mortem material has been utilized, and the glycogen distribution studied in the various tissues, particularly

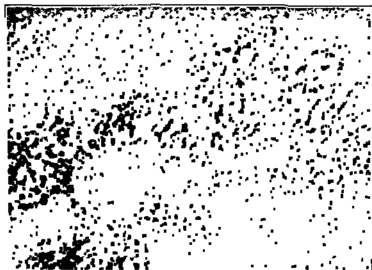


Fig. 41.—Moderate amount of glycogen in cells of periportal zone of liver. No glycogen in liver cell nuclei. The effect of insulin treatment. DA27 34. Female aged seventy three years. Duration of diabetes nine years. Best's carmine stain × 95

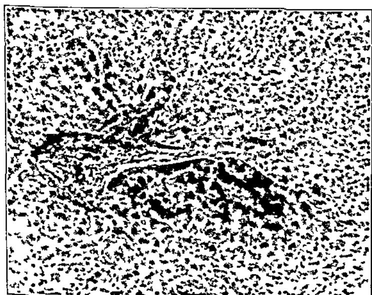


Fig. 42.—Slight amount of glycogen restricted to liver cells of periportal zones. No 4606. Female aged sixty-seven years. Duration of diabetes two years. Best's carmine stain × 95



FIG. 39.—Glycogenic infiltration (dark masses) in liver cell nuclei. Note very slight amount of glycogen in liver cells. Fifty minutes post mortem. DA27 36. Male aged sixty six years. Duration of diabetes seven months. × 150.

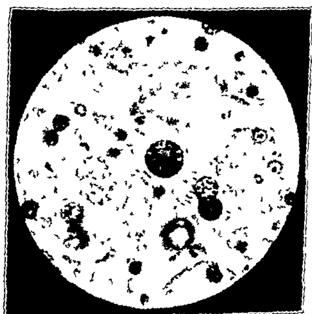


FIG. 40.—Glycogenic infiltration of liver-cell nuclei. Liver cells devoid of glycogen. Best's carmine stain. A22–44. Male aged seventy four years. Duration of diabetes eight years. × 500. (Joslin.)

not only on glycogen formation by the nucleus but also on the permeability of the nuclear membrane for glycogen

TABLE 12 —GLYCOGEN CONTENT OF LIVER-CELL NUCLEI

| Type of treatment | None | | Slight | | Moderate | | Marked | |
|---|---|---|---|---|---|---|---|---|
| | No | % | No | % | No | % | No | % |
| No insulin | 8 | 21 | 13 | 34 | 8 | 21 | [illegible] | 24 |
| Insulin | 28 | [illegible] | 11 | 26 | 3 | 7 | 1 | 2 |
| Insulin infection present | 6 | 57 | [illegible] | 19 | 18 | 18 | 6 | 6 |
| Control (nondiabetics) | 16 | [illegible] | [illegible] | 31 | 3 | 10 | 1 | 4 |

Glycogen is most marked in those cells in the periportal region Frequently the cells about the central vein contain none although it is present elsewhere in the lobule in large amounts

There is no antagonism between the deposition of fat and glycogen both being often present in the same cells in large amounts The histological foundation for the rather prevalent belief in this antagonism is probably the tendency of glycogen to be deposited at the periphery of the lobule first and fat to be most prominent about the central vein However these dis

rendered invisible in these cells with an adequate blood supply whereas the cells about the central veins cells which have to sit at second table may be fairly easily loaded with fat which they cannot metabolize and which therefore remains in visible form within the cells

For some reason the rabbit, in contrast to the human shows the glycogen deposits to be chiefly in the central portion of the lobule [22, 23] In the dog an even distribution through the lobule is the rule but in hepatic injury the deposition is most marked about the central veins [24]

Peculiarly enough liver glycogen seems to be increased in the alloxan diabetic rat [25, 26] The explanation is obscure Overactivity of the adrenal cortex may be partly responsible [27]

Estimations of liver glycogen vary greatly Eppinger[28] gives the possible amount of glycogen in the liver of a well nourished man as 400 grams (20 to 25 per cent) Dudley and Marrian[29] found 5.5 per cent in rabbits and Fisher and Lackey[30] 4 6 per cent in fed and 0 3 per cent in fasting dogs

TABLE 13 GLYCOGEN CONTENT OF LIVER CELLS

| Type of treatment | None | | Slight | | Moderate | | Marked | |
|---|---|---|---|---|---|---|---|---|
| | No | % | No | % | No | % | No | % |
| No insulin | 9 | 20 | 11 | 35 | 8 | 20 | 3 | 10 |
| Insulin | | | 8 | 24 | 12 | 30 | 13 | 40 |
| Insulin infection present | 7 | 8 | 20 | 31 | 32 | 38 | 20 | 23 |
| Control (nondiabetics) | 1 | 3 | 10 | 35 | 14 | 18 | 4 | 14 |

It seems probable that the hearts of infants of diabetic mothers also tend to have a higher than normal content of glycogen (*see* Table 41, page 252)

*Liver* Since glycogenic distention of the nuclei of liver cells is found in many other conditions than diabetes, this cannot be regarded as of particular importance However, in diabetic patients there is a very definite reciprocal relationship between the amount of intranuclear and the amount of intracytoplasmic glycogen When intranuclear glycogen deposition is marked there is but little or even no glycogen in the liver cells in these cases Conversely when there is much glycogen stored in the cytoplasm, there are few or no nuclei containing it



Fig 43 —Liver showing mo to periportal zone No glycog 1020 Male aged fifty years stain × 25

regard the vacuolation as a reliable index of glycogen

Eger and Klarner[21] maintain that glycogen is *formed* in the nucleus of the liver cell and then deposited or stored in the cytoplasm The accumulation in the nucleus in diabetes is regarded as indicating that insulin has an effect

Hildes *et al.*,[31] studied a number of normal controls as well as diabetics using both chemical and histological (Best's carmine) methods. In liver biopsies they found variations in 10 normals from 0.95 to 4.1 per cent with a mean of 2.15 per cent, and in 10 diabetics from 0.88 to 2.98 per cent with a mean of 1.84 per cent. In muscle biopsies (pectoralis major) they found 15 normals ranging from 1.13 to 3.89 per cent with a mean of 2.20 per cent and 6 diabetics from 0.72 to 2.49 per cent with a mean of 1.59 per cent. They did not consider the slightly lower values in the diabetics as significant. They found no correlation of the glycogen values with type of diabetes, fasting blood sugar level, or degree of ketosis.

*Pancreas.* In 8 instances considerable amounts of glycogen and in 12 instances slight amounts were found in the epithelium of the pancreatic ducts, both the smaller ducts with cuboidal epithelium and the larger ducts with columnar. Ohohashi[35] found this distribution in mammalian fetuses. From the recent work of Toreson as cited more fully in Chapters 3 and 27 it appears that glycogen may be found in the β cells of the islands (as well as in the ducts) in diabetes and may be associated with "hydropic change," which he prefers to call glycogen infiltration. Further work will be required to decide how frequently glycogen is to be found in this situation. One wonders whether it indicates cell injury or represents a storage phenomenon as in other tissues. Duff and Toreson[36] were able to prevent or reverse "hydropic change" in alloxan-diabetic rabbits with small doses of insulin in spite of continued hyperglycemia. They suggest that hypoinsulinemia is more important in the pathogenesis of this lesion than is hyperglycemia. Toreson[18] makes the interesting suggestion that it may be a sign of regeneration, thus correlating with the glycogen in the ducts from which new islands are usually formed. Glycogen infiltration of the islands can be produced in rabbits with large doses of cortisone (Figs. 13 and 14).

TABLE 11. GLYCOGEN CONTENT OF ISLAND BETA CELLS

| Type of treatment | None | | Slight | | Moderate | | Marked | |
|---|---|---|---|---|---|---|---|---|
| | No. | % | No. | % | No. | % | No. | % |
| No insulin | 4 | 27 | 1 | 10 | 3 | 20 | 2 | 11 |
| Insulin | 5 | 100 | | | | | | |
| Insulin, infection present | 11 | 55 | 6 | 40 | 3 | 15 | | |
| Control (nondiabetics) | 25 | 100 | | | | | | |

*Kidneys.* Insulin treatment definitely reduces the amount of glycogen present in the kidneys, as Root and Warren[37] pointed out. In many cases glycogen is entirely absent. In many others it is considerably reduced over that found in cases of diabetes not treated with insulin. Although Henle's loops are the most typical loci for glycogenic infiltration, the convoluted tubules as well are occasionally involved. This latter localization is sometimes named after Ebstein,[38] who noted the deposits but considered them to be necrosis of the cells of the tubules. Glycogenic granules may even

feeding dogs a diet similar to Voit's standard diet for man Junkersdorf obtained a value of 6 1 per cent. A maximum of 4 4 per cent was attained by Salter's[1] mice.

The above table gives evidence of the effect of insulin in promoting the deposit of glycogen in the liver, although this effect is somewhat lessened in septic cases. In severe diabetics without insulin even chemical examination may show no or very little glycogen in the liver.[11] The one nondiabetic liver showing no glycogen histologically was a case of healed toxic sclerosis with jaundice. What bearing this may have on the clinical finding that

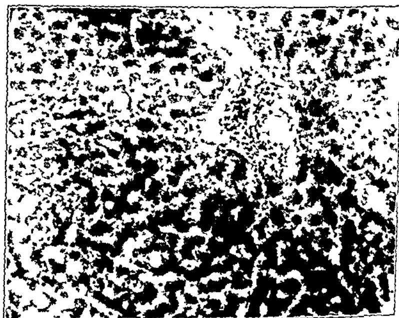


Fig. 44. Liver showing marked glycogenic infiltration of liver cells. Effect of insulin treatment. Also some fatty infiltration. DA[illegible] 71. Female, aged sixty years. Duration of diabetes 1 2 years. Best's carmine stain. × 150

cases of cirrhosis of the liver and diabetes do not do so well under insulin treatment as other cases is uncertain, but it suggests that there may be impairment of carbohydrate storage in cirrhosis. Ravdin[24] has summed up the evidence in favor of this view and concludes that injured liver cells have decreased ability to synthesize and store glycogen.

Recently a few studies of liver glycogen have been made in the living patient by needle biopsy. Bondy and Sheldon[27] in a case of diabetic coma which had been untreated for ten hours found only a trace of glycogen in a liver biopsy. After seven and one-half hours of treatment there was marked restoration of glycogen but fat vacuoles were numerous. Six days later there was a slight further increase in glycogen but no change in the fat

Hildes *et al.*,[34] studied a number of normal controls as well as diabetics using both chemical and histological (Best's carmine) methods. In liver biopsies they found variations in 10 normals from 0.95 to 4.1 per cent with a mean of 2.15 per cent, and in 10 diabetics from 0.88 to 2.98 per cent with a mean of 1.84 per cent. In muscle biopsies (pectoralis major) they found 15 normals ranging from 1.13 to 3.89 per cent with a mean of 2.20 per cent and 6 diabetics from 0.72 to 2.49 per cent with a mean of 1.59 per cent. They did not consider the slightly lower values in the diabetics as significant. They found no correlation of the glycogen values with type of diabetes, fasting blood sugar level, or degree of ketosis.

*Pancreas.* In 8 instances considerable amounts of glycogen and in 12 instances slight amounts were found in the epithelium of the pancreatic ducts, both the smaller ducts with cuboidal epithelium and the larger ducts with columnar. Oholashi[35] found this distribution in mammalian fetuses.

From the recent work of Toreson,[18] cited more fully in Chapters 3 and 25, it appears that glycogen may be found in the β cells of the islands (as well as in the ducts) in diabetes, and may be associated with "hydropic change," which he prefers to call glycogen infiltration. Further work will be required to decide how frequently glycogen is to be found in this situation. One wonders whether it indicates cell injury or represents a storage phenomenon as in other tissues. Duff and Toreson[36] were able to prevent or reverse "hydropic change" in alloxan-diabetic rabbits with small doses of insulin in spite of continued hyperglycemia. They suggest that hypoinsulinemia is more important in the pathogenesis of this lesion than is hyperglycemia. Toreson[18] makes the interesting suggestion that it may be a sign of regeneration, thus correlating with the glycogen in the ducts from which new islands are usually formed. Glycogen infiltration of the islands can be produced in rabbits with large doses of cortisone (Figs. 13 and 14).

TABLE 11. GLYCOGEN CONTENT OF PANCREATIC DUCT CELLS

| *Type of treatment* | *None* | | *Slight* | | *Moderate* | | *Marked* | |
|---|---|---|---|---|---|---|---|---|
| | No. | % | No. | % | No. | % | No. | % |
| No insulin | 4 | 27 | 6 | 40 | 3 | 20 | 2 | 13 |
| Insulin | 5 | 100 | | | | | | |
| Insulin, infection present | 11 | 55 | 6 | 30 | 3 | 15 | | |
| Control (non-diabetics) | 25 | 100 | | | | | | |

*Kidneys.* Insulin treatment definitely reduces the amount of glycogen present in the kidneys, as Root and Warren[37] pointed out. In many cases glycogen is entirely absent. In many others it is considerably reduced over that found in cases of diabetes not treated with insulin. Although Henle's loops are the most typical loci for glycogenic infiltration, the convoluted tubules as well are occasionally involved. This latter localization is sometimes named after Ebstein,[38] who noted the deposits but considered them to be necrosis of the cells of the tubules. Glycogenic granules may even

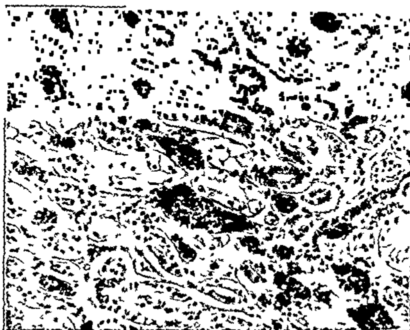


Fig 45 —Kidney showing marked glycogenic vacuolization of epithelium of convoluted tubules so-called Ebstein degeneration No 5151 Female aged fifty six years Duration of diabetes ten years Phosphotungstic acid hematoxylin stain × 225

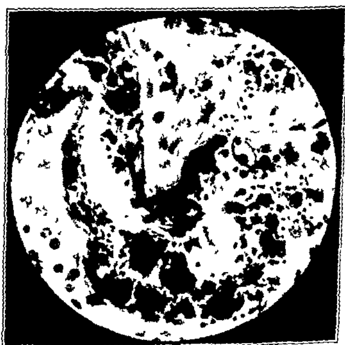


Fig 46 —Kidney showing marked glycogen deposition in epithelium of Henle's loop A22-44 Male aged seventy four years Duration of diabetes eight years Best's carmine stain × 560

be demonstrated within the capsular space but these probably are artifacts due to post mortem diffusion

Table 15 — Glycogen Content of Kidney

| Type of treatment | None | | Slight | | Moderate | | Marked | |
|---|---|---|---|---|---|---|---|---|
| | No | % | No | % | No | % | No | % |
| No insulin | 6 | 12 | 8 | 16 | 10 | 20 | 26 | 52 |
| Insulin | 15 | 35 | 15 | 35 | 9 | 21 | 4 | [illegible] |
| Insulin infection present | 21 | 23 | 28 | 27 | 38 | [illegible] | 13 | 11 |
| Control (nondiabetics) | 18 | 9[illegible] | 1 | 5 | | | | |

Practically every case of active diabetes either untreated or treated in the pre-insulin days showed glycogenic infiltration of the epithelium of Henle's loops at autopsy. Its absence in many cases treated with insulin shows that it is not an essential lesion of diabetes. This is also indicated by its presence in phlorhizin diabetes. Thus von Gierke states it has been found

1 In diabetes mellitus
2 After experimental extirpation of the pancreas
3 After extirpation of the celiac plexus
4 In phlorhizin poisoning

In the first three instances the blood sugar level is increased, in the last normal or low. The one point in common is the presence of glucose in the urine.

Since in the great majority of cases glycosuria in the human means diabetes, the presence of glycogen in Henle's loops may for all practical purposes be interpreted as an indication of active diabetes.

It is rather interesting that Ohobashi found that the distribution of glycogen in the kidney of the diabetic human is exactly the same as that in the normal kidney of the Anura. (See also page 168.)

Hinden has demonstrated glycogen in the collecting tubules of the kidney in the newborn of four species (rabbit, guinea pig, rat and man).

Loeschcke gives the following table based on 6 cases of diabetes showing the glycogen present elsewhere in the kidneys besides Henle's loops.

Table 16 — Amount of Glycogen in Renal Epithelium (Loeschcke)

| Case | Glomeruli | Convoluted tubules | Henle's loops |
|---|---|---|---|
| I | +++ | +++ | ++ |
| II | ++ | + | +++ |
| III | + | + | ++ |
| IV | − | + | ++++ |
| V | − | − | + |
| VI | − | − | − |

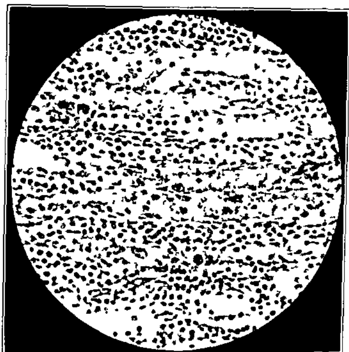


Fig. 47.—Henle's loops of the kidney showing vacuolization and glycogen granules. M886. Male aged fifty-four years. Duration of diabetes thirty years. Best's carmine stain. × 170



Fig. 48.—Glycogenic infiltration of epithelium of convoluted tubules. No. 5153. Female aged fifty-six years. Duration of diabetes ten years. Best's carmine stain. × 150

Rosenberg also mentions a case in which glycogen was present in the glomeruli and the epithelium of the convoluted tubules as well as in Henle's loops. Certain of the cases summarized in this series have shown a similar distribution of the glycogen. Sandmeyer gives a quantitative determination of the glycogen present in the kidney of a girl aged nine years who had had diabetes for two years: 0.1155 gram per 100 grams of kidney substance. The liver contained 0.6130 gram per 100 grams, or nearly six times as much.

There is close correspondence between increase in liver-cell glycogen and decrease in renal glycogen, and a similar relationship between liver-cell glycogen and liver nucleus glycogen. Thus, as normal storage increases under the influence of insulin, pathological storage decreases. This holds true in other organs as well. A diabetic responding satisfactorily to insulin treatment and dying of some condition other than diabetes or sepsis, such as cerebral hemorrhage, shows very nearly the same distribution of glycogen as does the normal individual.

In some cases it has been possible to correlate with the glycogen deposits in kidney and liver the blood and urine sugar levels obtained within a few hours before death. Typical results are shown in Table 17.

TABLE 17.—RELATION OF GLYCOGEN TO BLOOD AND URINE SUGAR

| Case No. | Insulin | Blood sugar mg. per cent | Urine sugar per cent | Liver glycogen: Cells | Liver glycogen: Nuclei | Kidney glycogen: Henle's loops | Kidney glycogen: Convoluted tubules |
|---|---|---|---|---|---|---|---|
| DA26 53 | + | 440 | 0.2 | + | + | + | |
| DA27 12 | + | 480 | 1.1 | ++ | + | + | − |
| DA27 33 | + | 5.0 | 5.5 | +++ | + | ++ | |
| DA27 31 | + | 800 | 1.0 | +++ | ++ | + | + |
| 88? | + | 180 | 0.0 | +++ | + | − | |
| 1603 | − | 300 | | + | ++ | ++ | + |
| 3 0? | + | 280 | 0.8 | +++ | + | ++ | − |
| 5112 | − | 140 | 0.1 | + | +++ | ++ | + |
| 5?6? | + | | 6.2 | ++ | | +++ | ++ |
| 5 13 | + | 320 | 2.5 | + | ++ | +++ | + |

There is a fair degree of correspondence between the urine sugar and the amount of glycogenic infiltration of the kidney, considering that variations in urine sugar must occur rather more rapidly than variations in glycogen content of the renal epithelium.

These results have received experimental confirmation in the work of Curtis *et al.* and Robbins. They studied the glycogen content of the renal tubules in a group of rats made diabetic with alloxan. They found that the appearance of glycogen in the renal tubules depended solely upon the terminal blood sugar levels of the animals. A value of 350 mg. per cent was the critical level above which glycogen was almost invariably de-

monstrable With terminal blood sugar levels below 300 mg per cent no glycogen was found in the kidneys

*Voluntary muscles* Many specimens of muscle came from legs amputated for gangrene They therefore may possibly be of dubious value owing to the influence of an impaired circulation which would certainly interfere with normal cellular function and might well influence the function of glycogen storage However, the muscle was always taken from as near the site of amputation as was possible without obtaining traumatized tissue In this way healthy tissue could be obtained Thanks are due to Dr L S McKittrick for permitting the use of fresh surgical material from his cases In the autopsy material portions of the pectoralis major were utilized

TABLE 18 —GLYCOGEN CONTENT OF VOLUNTARY MUSCLE

| | None | | Slight | | Moderate | | Marked | |
|---|---|---|---|---|---|---|---|---|
| *Type of treatment* | *No* | *%* | *No* | *%* | *No* | *%* | *No* | *%* |
| No insulin | | | 1 | 14 | 5 | 72 | 1 | 14 |
| Insulin | 1 | 8 | 3 | 23 | 9 | 69 | | |
| Insulin infection present | 1 | 1 | 29 | 39 | 30 | 40 | 15 | 20 |
| Control (nondiabetics) | | | 7 | 26 | 12 | 44 | 8 | 30 |

Although the results summarized in Table 18 are perhaps not statistically significant there is a tendency for decrease in muscle glycogen in the cases of diabetes as would be expected from our knowledge of the disease

Muscle glycogen has not so great significance for the diabetic as liver glycogen since only liver glycogen is directly transformable into glucose available for use of all the cells of the body [47]

*Skin* Unna[48] has called attention to the presence of glycogen in normal skin Recent work suggests that relatively little is present under usual circumstances

When it is present the epithelium of the stratum corneum sweat glands sebaceous glands and hair follicles contains most Becker[49] speaks of much glycogen in the duct cells of sweat glands in newborn infants In a few cases of diabetes the erector pili muscles show heavy deposits when the stores elsewhere in the skin are almost completely depleted Fichera[50] states that in the depancreatized animal all glycogen disappears except traces in stratified epithelium and in cartilage Both these tissues are avascular

Certain of the histological preparations in the earlier series (S W ) were paralleled by chemical determinations carried out by Trimble and Carey [5] They found the average sugar content (total reducing substances) of blood muscle and skin to be in nondiabetics 126 61 and 69 mg per cent and in diabetics 238 83 and 163 mg per cent respectively Changes in blood sugar were much more closely followed by skin sugar than by muscle sugar

It is perhaps premature to attempt to correlate variations in cutaneous

sugar and glycogen with the well known susceptibility of the diabetic patient to cutaneous infections. One of us (S. W.) has seen several instances of skin and muscle infection with E. coli in which there was sufficient production of gas from the carbohydrate present in the tissues to produce crepitation and so simulate gas bacillus infection. Two of these cases resulted fatally, and bacteriological studies both post mortem and ante mortem showed only coliform bacilli and staphylococci to be present. This type of infection we have never seen in nondiabetic patients. Curiously enough the original injury and infection of the skin in both these cases resulted from scratching incident to pruritus, showing this may be a dangerous as well as an annoying symptom.

*Brain.* Brain tissue does not lose the power of converting glucose to lactic acid in diabetes. But little of the lactic acid produced is formed from glycogen, most being formed directly from blood sugar.[52] What little glycogen is present in the brain is probably immobilized, as it does not fall following pancreatectomy.[53] Kerr and Ghantus[54] found an average of 0.1 per cent in dog brain and 0.08 per cent in rabbit brain. Munzer[55] demonstrated stainable glycogen in human retina, ependyma, chloroid plexus, neurohypophysis. In the present series, glycogen has been found once in the cortical cells (No. 13224), occasionally in the ependyma and choroid. [illegible][56] found glycogen in the cortical cells of 14 out of 19 cases dying of diabetic coma.

The pigmented iris epithelium of the eye may contain large quantities of glycogen associated with the vacuolated appearance of the cells with considerable fragility of the iris, a fact well known to ophthalmic surgeons (see Chapter 13).

*Pituitary.* Medwedeff[57] noted much glycogen in the anterior pituitary, particularly of 3 cases. Munzer[55] noted some in the neurohypophysis. Stains for glycogen were not done in the present series, as the material was used for serial sections.

*Placenta.* Examination of placenta from diabetic mothers has revealed no significant difference in glycogen storage from that of normal mothers. (See also Chapter 23.)

*Leukocytes.* Glycogenic granules were found in the leukocytes of the granulocytic series in 18 cases of diabetes, all of which showed extensive deposits elsewhere typical of diabetes. This finding is less significant as

TABLE 11. GLYCOGEN CONTENT OF LEUKOCYTES

| *Type of treatment* | *None* | | *Slight* | | *Moderate* | | *Marked* | |
|---|---|---|---|---|---|---|---|---|
| | No. | % | No. | % | No. | % | No. | % |
| No insulin | 1 | – | [illegible] | 33 | 3 | 20 | [illegible] | 40 |
| Insulin | 1 | 10 | [illegible] | 30 | | | | |
| Insulin [illegible] | 3 | 9 | 25 | 75 | 1 | 12 | 1 | 3 |
| Control (nondiabetics) | 2 | 20 | 8 | 80 | | | | |

7

monstrable With terminal blood sugar levels below 300 mg per cent no glycogen was found in the kidneys

*Voluntary muscle* Many specimens of muscle came from legs amputated for gangrene They therefore may possibly be of dubious value owing to the influence of an impaired circulation which would certainly interfere with normal cellular function and might well influence the function of glycogen storage However the muscle was always taken from as near the site of amputation as was possible without obtaining traumatized tissue In this way healthy tissue could be obtained Thanks are due to Dr I S McKittrick for permitting the use of fresh surgical material from his cases In the autopsy material portions of the pectoralis major were utilized

TABLE 18 —GLYCOGEN CONTENT OF VOLUNTARY MUSCLE

| | None | | Slight | | Moderate | | Marked | |
|---|---|---|---|---|---|---|---|---|
| *Type of treatment* | *No* | *%* | *No* | *%* | *No* | *%* | *No* | *%* |
| No insulin | | | 1 | 14 | 5 | 72 | 1 | 14 |
| Insulin | 1 | 8 | 3 | 23 | 9 | 69 | | |
| Insulin infection present | 1 | 1 | 29 | 39 | 30 | 40 | 15 | 20 |
| Control (nondiabetics) | | | 7 | 26 | 12 | 44 | 8 | 30 |

Although the results summarized in Table 18 are perhaps not statistically significant there is a tendency for decrease in muscle glycogen in the cases of diabetes as would be expected from our knowledge of the disease

Muscle glycogen has not so great significance for the diabetic as liver glycogen since only liver glycogen is directly transformable into glucose available for use of all the cells of the body [47]

*Skin* Unna[48] has called attention to the presence of glycogen in normal skin Recent work suggests that relatively little is present under usual circumstances

When it is present the epithelium of the stratum corneum sweat glands sebaceous glands and hair follicles contains most Becker[49] speaks of much glycogen in the duct cells of sweat glands in newborn infants In a few cases of diabetes the erector pili muscles show heavy deposits when the stores elsewhere in the skin are almost completely depleted Fichera[50] states that in the depancreatized animal all glycogen disappears except traces in stratified epithelium and in cartilage Both these tissues are avascular

Certain of the histological preparations in the earlier series (S W ) were paralleled by chemical determinations carried out by Trimble and Carey[5]

s of blood

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food

sugar were much more closely followed by skin sugar than by muscle sugar

It is perhaps premature to attempt to correlate variations in cutaneous

sugar and glycogen with the well-known susceptibility of the diabetic patient to cutaneous infections. One of us (S. W.) has seen several instances of skin and muscle infection with *E. coli* in which there was sufficient production of gas from the carbohydrate present in the tissues to produce [illegible]

type of infection we have never seen in nondiabetic patients. Curiously enough the original injury and infection of the skin in both these cases resulted from scratching incident to pruritus, showing this may be a dangerous as well as an annoying symptom.

*Brain.* Brain tissue does not lose the power of converting glucose to lactic acid in diabetes. But little of the lactic acid produced is formed from glycogen, most being formed directly from blood sugar.[52] What little glycogen is present in the brain is probably immobilized, as it does not fall following pancreatectomy.[53] Kerr and Ghantus[54] found an average of 0.1 per cent in dog brain and 0.08 per cent in rabbit brain. Munzer[55] demonstrated stainable glycogen in human retina, ependyma, chloroid plexus [illegible] ogen has been found once [illegible] ie ependyma and choroid [illegible] l out of 19 cases dying of diabetic coma.

*Eye.* The pigmented iris epithelium of the eye may contain large quantities of glycogen associated with the vacuolated appearance of the cells and with considerable fragility of the iris, a fact well known to ophthalmic surgeons (*see* Chapter 13).

*Pituitary.* Medwedeff[57] noted much glycogen in the anterior pituitary particularly of 3 cases. Münzer[5] noted some in the neurohypophysis. Stains for glycogen were not done in the present series as the material was used for serial sections.

*Placenta.* Examination of placentae from diabetic mothers has revealed no significant difference in glycogen storage from that of normal mothers (*See also* Chapter 23.)

*Leukocytes.* Glycogenic granules were found in the leukocytes of the granulocytic series in 18 cases of diabetes, all of which showed extensive deposits elsewhere typical of diabetes. This finding is less significant as

TABLE 11. GLYCOGEN CONTENT OF LEUKOCYTES

| | *None* | | *Slight* | | *Moderate* | | *Marked* | |
|---|---|---|---|---|---|---|---|---|
| *Type of treatment* | *No* | *%* | *No* | *%* | *No* | *%* | *No* | *%* |
| No insulin | 1 | [illegible] | 5 | 33 | 3 | 20 | 6 | 40 |
| Insulin | 1 | 10 | 9 | 90 | | | | |
| Insulin, infection present | 3 | 9 | 25 | 76 | 4 | 12 | 1 | 3 |
| Control (nondiabetics) | 2 | 20 | 8 | 80 | | | | |

7

monstrable With terminal blood sugar levels below 300 mg per cent no glycogen was found in the kidneys

*Voluntary muscles* Many specimens of muscle came from legs amputated for gangrene They therefore may possibly be of dubious value owing to the influence of an impaired circulation which would certainly interfere with normal cellular function and might well influence the function of glycogen storage However, the muscle was always taken from as near the site of amputation as was possible without obtaining traumatized tissue In this way healthy tissue could be obtained Thanks are due to Dr I S McKittrick for permitting the use of fresh surgical material from his cases *In the autopsy material portions of the pectoralis major were* utilized

TABLE 18 —GLYCOGEN CONTENT OF VOLUNTARY MUSCLE

| | *None* | | *Slight* | | *Moderate* | | *Marked* | |
|---|---|---|---|---|---|---|---|---|
| *Type of treatment* | *No* | *%* | *No* | *%* | *No* | *%* | *No* | *%* |
| No insulin | | | 1 | 14 | 5 | 72 | 1 | 14 |
| Insulin | 1 | 8 | 3 | 23 | 9 | 69 | | |
| Insulin infection present | 1 | 1 | 29 | 39 | 30 | 40 | 15 | 20 |
| Control (nondiabetics) | | | 7 | 26 | 12 | 44 | 8 | 30 |

*Although the results summarized in Table 18 are perhaps not* statistically significant there is a tendency for decrease in muscle glycogen in the cases of diabetes as would be expected from our knowledge of the disease

Muscle glycogen has not so great significance *for the diabetic* as liver glycogen since only liver glycogen is directly transformable into glucose available for use of all the cells of the body [47]

*Skin* Unna[48] has called attention to the presence of glycogen *in normal* skin Recent work suggests that relatively little is present under usual circumstances

When it is present the epithelium of the stratum corneum sweat gland sebaceous glands and hair follicles contains most Becker[49] speaks of much glycogen in the duct cells of sweat glands in newborn infants In a few cases of diabetes the erector pili muscles show heavy deposits when the *stores elsewhere in the skin are almost completely depleted Fichera[50] states that in the depancreatized animal* all glycogen disappears except traces in stratified epithelium and in cartilage Both these tissues are avascular

Certain of the histological preparations *in the earlier* series (S W) were paralleled by chemical determinations carried out by Trimble and Carey [?] They found the average sugar content (total reducing substances) of blood

[illegible] per cent and in

blood

ugar

ieus

It is perhaps premature to attempt to correl[illegible]

sugar and glycogen with the well known susceptibility of the diabetic patient to cutaneous infections. One of us (S. W.) has seen several instances of skin and muscle infection with *E. coli* in which there was sufficient production of gas from the carbohydrate present in the tissues to produce crepitation and so simulate gas-bacillus infection. Two of these cases resulted fatally, and bacteriological studies both post mortem and ante mortem showed only coliform bacilli and staphylococci to be present. This type of infection we have never seen in nondiabetic patients. Curiously enough the original injury and infection of the skin in both these cases resulted from scratching incident to pruritus, showing this may be a dangerous as well as an annoying symptom.

*Brain.* Brain tissue does not lose the power of converting glucose to lactic acid in diabetes. But little of the lactic acid produced is formed from glycogen, most being formed directly from blood sugar.[52] What little glycogen is present in the brain is probably immobilized, as it does not fall following pancreatectomy.[53] Kerr and Ghantus[54] found an average of 0.1 per cent in dog brain and 0.08 per cent in rabbit brain. Munzer[55] demonstrated stainable glycogen in human retina, ependyma, choroid plexus [illegible] lycogen has been found once [illegible] the ependyma and choroid [illegible] 14 out of 19 cases dying of diabetic coma.

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TABLE 1[illegible]. GLYCOGEN CONTENT OF LEUKOCYTES

| | *None* | | *Slight* | | *Moderate* | | *Marked* | |
|---|---|---|---|---|---|---|---|---|
| *Type of treatment* | *No.* | *%* | *No.* | *%* | *No.* | *%* | *No.* | *%* |
| No insulin | 1 | 7 | 5 | 33 | 3 | 20 | 6 | 40 |
| Insulin | 1 | 10 | 9 | 90 | | | | |
| Insulin, infection present | 3 | 9 | 25 | 76 | 4 | 12 | 1 | 3 |
| Control (nondiabetics) | 2 | 20 | 8 | 80 | | | | |

7

*most of the cases were ones with evidence of sepsis Glycogen is usually* present in pus cells

*Other tissues* Medwedeff[57] and Geipel[58] record the occasional presence of glycogen in various tissues *e g* thymus

**Value in Diagnosis** —The value of this means of histological diagnosis is well shown by a case (No 3928) where the existence of diabetes was established by the abnormal glycogen distribution Miss A P aged sixty nine years was supposed to have had mild diabetes but the diagnosis was never definitely established In November 1928 she developed a left hemiplegia and gradually grew worse with mental deterioration She showed no sugar in her urine but this was probably due to the low caloric intake which was little over that which would be allowed a moderately severe diabetic Death occurred February 9 1929 and an autopsy was performed three hours post mortem

The chief cause of death was multiple foci of necrosis of the cerebrum secondary to arteriosclerosis There was chronic pancreatitis with sclerosis and hyalinization of the islands of Langerhans

Best s carmine stain showed a slight amount of glycogen in the heart and voluntary muscle a trace in the liver cells much in the liver-cell nuclei and a moderate amount in the epithelium of Henle s loops On these findings a diagnosis of diabetes mellitus was made

Not only is the glycogen distribution of considerable aid in establishing a diagnosis of [illegible] As an example Case [illegible] entered the hospital un[illegible] allowing a gradual downhill course of two years duration with relatively little food intake She had been advised to enter the hospital several times previously but refused until practically *in extre*[illegible] sentially negative except for weakne[illegible] was found in her possession and she [illegible] amounts for the past two years December 10 at 11 A M Cheyne-Stokes respirations were noticed her blood pressure was too low to read her pupils were still contracted The nurse stated that during the past three and a half weeks the patient had not taken more than a small fraction of a maintenance diet December 10 at 11 30 A M 1000 cc of hypertonic glucose solution were given subcutaneously Subsequently a catheter specimen of urine showed 2 per cent sugar but no acetone or diacetic acid with blood sugar of 670 mg per cent No previous urine specimen had been examined owing to her incontinence of urine and feces The finding of this urine sugar led to the suspicion of diabetes Death occurred on December 10 at 9 10 P M

However at autopsy twelve hours afterward the pancreas was found to be normal there was abundant glycogen in the liver and in the muscles there was no glycogen in the liver cell nuclei or in the kidneys In other words the glycogen distribution was that of a normal individual not of a

diabetic. The probability is that the absorbed glucose could not be metabolized by an individual so near death. The absence of glycogen in the epithelium of the kidney tubules in the presence of a recent glycosuria is probably due to the same fact. The length of time post mortem cannot explain the absence of glycogen here, as there was no vacuolization of the renal epithelium which persists, is presumptive evidence of the presence of glycogen at the time of death until disintegration of the cells. Moreover normal glycogen deposits were still in evidence.

The known value of glycogen to the diabetic patient and the striking evidence of the part insulin plays in the deposition of glycogen adduced by these histological studies emphasize the importance of an adequate dosage of insulin. The necessity of preventing infections in diabetic patients is strikingly brought out by the decrease of glycogen storage in insulin treated cases when sepsis appears. This of course parallels the long recognized observation that tolerance drops during infections.

**Glycogen Storage Disease.**—Glycogen storage disease with hepatomegaly as one of the outstanding physical signs is most puzzling. Von Gierke[58] reported a case with 10.4 per cent glycogen in the liver and 6 per cent in the kidneys. Lusheim[59] one with 14 per cent liver glycogen. Although death usually occurs during childhood and diabetes is not present ordinarily, cases have been reported which might be explained by diabetes developing in glycogen storage disease, such as those of Parnas and Wagner,[60] Gjurić,[61] Bruin and co-workers, and Stetson and Ohler.[62] However these cases leave something to be desired in the way of completeness.

Wachstein[63] has reported a case of glycogen storage disease with marked hyperplasia of the islands of Langerhans and has in addition made a careful study of the distribution of acid and alkaline phosphatase which he found to be not significantly different from the normal. It is by no means clear whether this disease represents a defect in the glycogenolytic enzyme system,[64] a pituitary hypothalamic dysfunction[65] or something else.[66] Possibly, as indicated by Bauer, it may be due to a deficiency of the newly postulated hyperglycemic factor of the α cells.

Landing and Bingle[67] point out interesting similarities between glycogen storage disease and diabetes, such as the familial occurrence in both, the occurrence of diabetes in the relatives of some patients with glycogen storage disease, and the alleged coexistence of the two diseases. However, data are still insufficient to indicate any fundamental relationship between the two diseases.

Galactose diabetes, a rare congenital metabolic disturbance, is apparently characterized not so much by glycogen storage as by fatty infiltration of the liver.[68]

## MUCOPOLYSACCHARIDES

Recent work suggests that the mucopolysaccharides, those ubiquitous substances which are components of the ground substance and basement

membranes of connective tissue and blood vessel walls may play an important role in disease states especially in those which appear to involve collagen primarily and in the so-called degenerative diseases Indeed from a broadly speculative point of view practically all the histological lesions associated with diabetes may be interpreted as representing a disturbance in metabolism or storage of polysaccharides either relatively simple one (glycogen) or more complex varieties (mucopolysaccharides) (See Chapter 28 for discussion of this point)

## SUMMARY

On the whole it may be said that there are many gaps in our knowledge of the histochemistry of carbohydrate metabolism especially as regard the localization of glycogen deposits Indeed the deposition of glycogen in such apparently bizarre and meaningless places as the iris epithelium of the eye nuclei of liver cells and cells of the islands of Langerhans is difficult to correlate with any known facts of carbohydrate metabolism

Letterer[69] has recently written an interesting review of the problem of storage and storage diseases He proposes the following classification based on von Gierke's term thesaurismosis (1) thesaurosis or physiological storage (2) thesauropathy or diseases of cells tissues or organs as a consequence of storage of a particular material i e true storage d ease and (3) patho thesaurosis or pathological storage in primary metabolic diseases (this he also calls Stapelungsdystrophie or deposition dystrophy) Presumably the peculiar glycogen deposits in diabetes mellitus come under the third classification

It is to be hoped that some of the newer methods[70,71] for histochemical demonstration of polysaccharides and enzymes may clarify some of the histological manifestations of aberrant carbohydrate metabolism

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## Chapter 6

# PATHOLOGICAL EVIDENCES OF ABNORMAL FAT METABOLISM

As noted in the preceding chapter old boundary lines between the metabolic cycles of carbohydrate fat and protein are being broken down by recent work which shows the essential interdependence of these processes However certain morphological accumulations of lipids may conveniently be studied under this heading

The frequent occurrence of lipemia and of hypercholesterolemia in diabetes has long been known and very carefully studied [1,2] Naturally less attention has been attracted by the rather rare abnormal deposition of lipoid material in various organs or rather in the reticulo-endothelial cells [3]

## I THE SPLEEN AND RETICULO ENDOTHELIAL SYSTEM

This deposition when present is more marked in the spleen than in other organs The rarity of the condition in its full blown state is striking It was encountered in only 13 out of 527 diabetic autopsies in the earlier series

Much of the following discussion of lipoid deposits in the spleen has been taken from an article by Root and Warren [4]

While in the average spleen removed at autopsy there is little evidence either gross or microscopic of lipoid content Poscharisky[5] demonstrated fat to be present in spleens removed from cases of many different diseases and to be more abundant in those spleens containing amyloid and hyaline masses This fat is present in small droplets and gives the characteristic staining reactions for neutral fat He further states that in children the fat is chiefly within the epithelioid cells of lymph nodules of the spleen while in adults it is found in the pulp trabeculae capsule and vessel wall

Kusunoki[6] added further proof of the frequent though inconspicuous occurrence of lipoid in the spleen and showed that most was contained in cells of the reticulum The lipoid gave the following staining reactions red with Sudan III black with osmic acid and blue or blue-violet with Nile blue sulphate Kusunoki also found the number and prominence of the lipoid-containing cells in the spleen to run parallel with the lipoid content of the blood

Aside from this fairly common finding of rare lipoid-containing cells in the spleen there are a few cases of diabetes with lipemia recorded in which very marked hyperplasia of these cells occurs and the lipoid present does not as a rule give the staining reactions usually encountered

The first instance of this striking change in the spleen was mentioned by Coats.[7] The patient, suffering from diabetes with lipemia, had died in coma. Large lipoid-containing cells made up most of the splenic tissue. Other cases have since been reported, and a general discussion is given by Schmidt.[8]

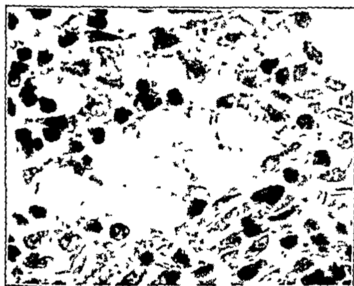


Fig. 49.—Lipoid histiocytosis of spleen. Case M. F. 8. Male, aged sixteen years. Duration of diabetes five and a half years. Death in coma with marked lipemia. Eosin methylene blue stain. × 500.

Several examples of this condition have been available for study in this laboratory, including material furnished through the kindness of Drs. F. B. Mallory, I. M. Rabinowitch and R. H. Jaffé.

It was noted in these cases that, while the spleen was most striking, the reticulo-endothelial cells elsewhere were involved. So far as can be judged from the staining reactions, the lipoid is present in various forms and sometimes even varies in the same case, though cholesterol esters or related substances predominate. Thus, in one case the larger droplets are presumably soaps or fatty acids, among which, according to the reaction with osmic acid, are unsaturated fatty acids. The smaller droplets contain cholesterol esters.

A case of generalized xanthomatosis was reported by Lubarsch[9] in a soldier aged twenty-six years, with diabetes of two years' duration, who developed a marked lipemia and enlargement of the liver late in the disease. At autopsy a small pancreas with atrophic islands was found, as well as widespread xanthomatous infiltration.

One of the puzzling features of this condition is the variation in chemical constitution of the lipoids taken up by the cells of the reticulo-endothelial system and the difference between these lipoids and those of the blood. Of course the staining reactions are not entirely reliable criteria but in several of the cases of this condition there is marked difference in the reactions of the lipoid material in the circulating blood and that present in the reticulo-endothelial system. The selective absorption of cholesterol compounds and phosphatides seems not impossible.

Anitschkow[10] produced a somewhat similar picture in the spleen of animals by experimental feeding with cholesterol.

In Schuller Christian's disease[11] cholesterol and its esters are deposited especially in the membranous bones. This has no definite association with diabetes mellitus. The lipoid stored in Gaucher's disease is chiefly kerasin.[12 13] In the lipoid histiocytosis of diabetes the spleen never attains the large size of Gaucher's disease.

The essential splenohepatomegaly of Niemann Pick must be distinguished from the lipoid cell hyperplasia encountered in diabetes. In this *former condition the lipoid laden cells are present in the pulp.*[14 15] Here the lipoids are chiefly phosphatides. The material present in the diabetic lipoid histiocytosis is somewhat different: cholesterol, fatty acids and neutral fats predominating with a small amount of phosphatides, if we may trust the rather uncertain results of staining reactions. An excellent review of the condition in diabetes is given by Smith.[16]

As one of us (S. W.) has previously pointed out, atheromata of the aorta are very *frequently* associated with this condition in *diabetes regardless of age*. Thus in the case of Oppenheimer and Ilhberg[17] atheromatous patches were found in the intima of the aorta and in the endocardium of a girl aged six years. Atheromata were found in the aorta of our Case I only sixteen years of age and our Case II, thirty three years of age, died of infarct of the heart resulting from marked atheromatosis and sclerosis of the coronary arteries with involvement of the aorta as well. Case III, aged twenty-one years, showed numerous atheromatous *patches in the* intima of the aorta as did Case IV, aged twelve years.

## II THE SKIN

The association of xanthoma of the skin and diabetes has long been known, xanthoma diabeticorum being a familiar phrase to every medical student.

It is important to distinguish between xanthelasma (xanthoma palpebrarum) and xanthoma diabeticorum.[18] Xanthelasma refers to benign *slowly growing flat yellowish tumors of the nasal portion of the eyelid* usually bilateral and not occurring much more frequently in diabetics than in the general population. Microscopically they are made up of fat-con

taining macrophages but may not show as many Touton giant cells * as other types of xanthoma Xanthoma diabeticorum which is called xanthoma eruptivum by some authors[20, 21] and not sharply distinguished from xanthoma tuberosum by others[22] is apparently definitely related to hyperlipemia Thannhauser[23] classifies it as secondary eruptive xanthoma associated with symptomatic hyperlipemia It occurs as a tumor like lesion often brilliant yellow associated with a red periphery which is characteristic The lesions may appear quite suddenly and are usually most numerous on the outside and back of the forearm and especially about the elbows and knees where they may be confluent Histologically they are indistinguishable from xanthoma tuberosum being made up of lipoid containing macrophages with occasional Touton giant cells They may however show more peripheral inflammatory reaction and more fibroblastic proliferation than xanthoma tuberosum and if biopsy is taken when the lesion is undergoing involution cholesterol and other lipoids may be found extracellularly as well as intracellularly (Figure 50 )

In this diabetic series true xanthomas are extremely rare in spite of the fact that hypercholesterolemia is fairly frequent[24] There appears to be considerable variation in the cholesterol fat ratio in these lesions

However occasional cases a section from one of which is shown in Figure 50 show greater relative amounts of cholesterol In this case cholesterol made up 45 per cent of the lipoid present though only 1 C per cent of the total mass Curiously enough the tumors in this patient disappeared as rapidly under dietary and insulin treatment as do the ordinary xanthomas

Examination of the eyelids by Waite and Beetham[25] showed 59 xanthelasmas among 4 001 diabetic eyes (1 5 per cent) and 7 among 914 nondiabetic eyes (0 8 per cent)

In 4 of Joslin s[19] 12 cases of xanthoma diabeticorum lipemia retinalis was also present Arcus shows no definite association with hypercholesterolemia

The highest blood cholesterol value associated with xanthoma found in this laboratory is 781 mg per 100 cc In practically all cases of xanthoma reduction of the fat in the diet has reduced the blood lipoid and has resulted in the disappearance of the xanthoma One of Wile s[26] cases was a diabetic When the patient was placed on a practically fat free low-calorie diet the multiple xanthomas which he had decreased markedly in size Later the patient was placed on a relatively high fat diet on which he became sugar free and the tumors showed an even more rapid disappearance On resumption of normal diet the xanthomas reappeared

The presence of true xanthoma is a strong hint that the diet needs revision from the standpoint of lowering the lipoid intake Adlersberg *et al*[27] believe that the tendency to xanthoma is inherited as an incomplete

* This is a giant cell in which the nuclei lie near the center of the cell grouped around a small island of nonfoamy cytoplasm and surrounded by foamy cytoplasm[18]

dominant trait in persons who carry two abnormal genes for cholesterol and that atherosclerosis is also frequent in these people.

Still another factor to be considered is that the diet of the average diabetic patient is rich in lipochromes and local cutaneous lipoid deposits may very well be emphasized by deposition in them of lipochromes. Connor[illegible] found the normal range of carotinemia to be from 0.0 to 0.07 mg. and he found in 18 of Joslin's diabetic patients selected at random values ranging from 0.05 to 0.16 mg. Miss Hunt in this laboratory has found in 19 determinations on diabetic patients who were clinically suspected of carotinemia values ranging from 0.09 to 0.86 mg. Whether carotin has anything to do with the peculiar yellowish color of the bones in some diabetics[29] is not clear.

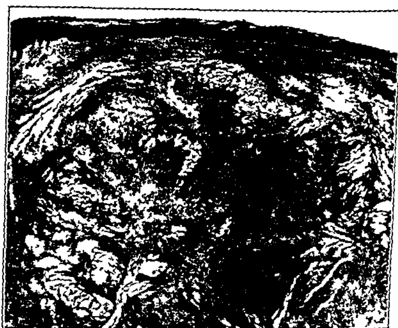


Fig. 50. Section of xanthoma of skin showing numerous masses of cholesterol crystals and [illegible] dense connective tissue. Male aged forty-eight years. Duration of diabetes eight years. Lesions similar to that above cleared up under insulin treatment. Hematoxylin eosin stain. × 20

Necrobiosis lipoidica diabeticorum was first described by Oppenheim[30] but named by Urbach.[31] Twenty-five cases, most of them young, occurred among Joslin's[18] patients.

The disease is not confined to diabetics, although about 90 per cent of the reported cases have been diabetics, mostly women and usually poorly controlled. According to Hildebrand *et al.*[32] the gross lesions may go through four phases: (1) a small reddish infiltrated papule, (2) an increasingly

rge lesion becoming violescent (3) a slightly raised firm yellow plaque which may soften and sometimes ulcerate and (4) a flattened yellowish region with central atrophy and depression and a peripheral irregular ling red or violescent region of infiltration. Microscopically there is robiosis of collagen with homogenization and degeneration of fibers. mon and Fisher[33] stress the pallor of the necrobiotic areas in the hema toxylin-eosin stain, also loss of elastic tissue and a variable peripheral cellular reaction sometimes having a tuberculoid appearance. The lipids which were not present in the necrobiotic areas in all their cases stained brownish red with Sudan III and were only rarely doubly refractile. The lipids are characteristically extracellular in this condition.

Calcification of the subcutaneous tissues[34] appeared in the legs of two elderly diabetic women. Calcium was present both in the fat and in the corium. Their blood chemical findings other than blood sugar were essentially normal. Diabetes had been present for fourteen and six years respectively. No explanation has been found.

## III THE GALL BLADDER

Inasmuch as the bile is the chief route for elimination of cholesterol from the body and many gall stones are of the cholesterol type, abnormalities of the gall bladder might be expected as a result of the disturbed lipoid metabolism in diabetes.* Table 20 shows the incidence of gall stones and cholecystitis in a group of 453 autopsied diabetics over thirty years of age and in 500 nondiabetic autopsies in the same age group.

TABLE 20.—FREQUENCY OF GALL BLADDER LESIONS IN AUTOPSIED CASES OVER THIRTY YEARS OF AGE

| | Diabetic | % | Nondiabetic | % |
|---|---|---|---|---|
| Number of cases | 453 | 100 | 500 | 100 |
| Cholelithiasis | 133 | 31 | 107 | 21 |
| Cholecystitis without stone | 28 | 6 | 14 | 3 |
| Previous operative removal of gall bladder (no stones) | 1 | 0.2 | 2 | 0.4 |
| Carcinoma | 1 | 0.2 | 0 | 0 |

TABLE 21.—RELATIVE INCIDENCE OF GALL STONES AT DIFFERENT AGES (OTHERS)

| Age, yrs | Number of cases | Percentage of total |
|---|---|---|
| 20 to 30 | 3 | 1.2 |
| 30 to 40 | 11 | 4.2 |
| 40 to 50 | 33 | 5.9 |
| 50 to 60 | 53 | 9.4 |
| 60 to 70 | 62 | 13.3 |
| 70+ | 36 | 19.1 |

* It is recognized that disturbed lipid metabolism may not be the only cause of the apparent increased incidence of gall bladder disease in diabetes. The susceptibility of diabetics to infection may also be an important factor. However, it is convenient to discuss the gall bladder in this chapter.

Table 21 from Ophuls[35] shows the incidence of gall stones by age groups in 3000 autopsies of which only 34 were performed upon diabetics

The available statistics as to the occurrence of gall stones vary greatly from 3 per cent in White's[36] series of 11 031 atuopsies based on Guy's Hospital material to 21 per cent in Mentzer's[37] series of 600 autopsies at the Mayo Clinic Kaufmann[38] at Basle found 10 9 per cent of 11 023 autopsies to show gall stones

Wilder[39] reported 16 cases of cholelithiasis and 4 of severe cholecystitis in 58 autopsies on diabetic patients performed at the Mayo Clinic or 28 per cent with cholelithiasis and 34 5 per cent with gall bladder disease

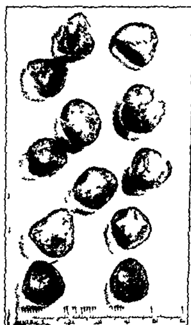


Fig. 51 —Gall stones largely cholesterol from Case DA27 95 Female aged sixty years Duration of diabetes five years These stones were found at autopsy There had been no definite symptomatology pointing toward them

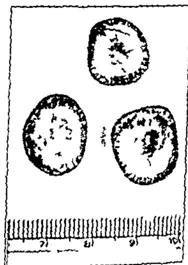



Fig 52.—Cross section of several of the stones shown in Figure 51 Slightly enlarged

This compares closely with our findings (31 per cent of 453 autopsies showing cholelithiasis and 36 per cent showing either cholelithiasis or cholecystitis)

This increased prevalence of cholelithiasis and cholecystitis in diabetes is of obvious importance from two angles the gall bladder disease in itself and the marked depression of sugar tolerance exerted by any inflammatory process in the diabetic organism

In view of the stress laid on gall bladder disease as a possible cause of pancreatic injury, and hence diabetes, it is of interest to consider whether diabetes or gall bladder disease appeared first in these cases. In most instances the onset of diabetes antedated the appearance of symptoms referable to the gall bladder disease. This of course is of little value as evidence as the stones were silent in many cases. Moreover Joslin's cases with more detailed clinical data showed the gall bladder disease to develop first. Relatively few of the cases examined post mortem show sufficient pancreatic change of inflammatory type to admit pancreatitis due to gall bladder disease as a cause of the diabetes, although practically all show some pancreatic fibrosis of slight to moderate degree.

In a few instances, as Case A51-17 (Peter Bent Brigham Hospital), the gall stones in all probability predisposed to repeated attacks of acute pancreatitis of mild type with eventually sufficient injury to the islands of Langerhans to permit diabetes to develop. This case occurred in a male aged fifty seven years. For five years attacks of pain in the right upper quadrant recurred at intervals with occasional periods of jaundice. One month before death from bronchopneumonia diabetes of moderate severity developed. At autopsy three large stones were found in a thick walled gall bladder. The pancreas showed patches of fat necrosis and fibrosis. The islands were hypertrophied.

However, cases of this type are rare. In Table 22 the occurrence of gall stones in the earlier series is compared in various periods of duration of the diabetic process.

TABLE 22.—RELATION OF GALL STONES TO DURATION OF DIABETES

| *Duration of diabetes, yrs* | *Per cent with gall stones* |
|---|---|
| 0 to 5 | 20.4 |
| 6 to 10 | 36.[illegible] |
| 11 to 15 | 41.3 |
| 15+ | 33.3 |
| ? | 21.2 |

Of course the known mildness of gall stone diabetes may account for the strikingly increased occurrence of gall stones in the cases of longer duration.

The association of cholelithiasis with arteriosclerosis is also marked. Of course this might well be explained by the tendency of both to occur in the upper age groups, but the strikingly high incidence of both these lesions in diabetes suggests a common origin possibly in the disordered fat metabolism.

Joslin[40] cites a striking case, No 310, who developed diabetes at the age of seventeen years. When the patient was only twenty-one years of age gallstones were definitely diagnosed. Death occurred in coma at thirty-eight years of age.

The bulk of stones found are of the cholesterol type, though occasionally the pigment-calcium stones are encountered. A few examples of analyses from a series of diabetic and nondiabetic gall stones performed by Dr. J. Du Vigneaud in this laboratory are given below and illustrate the marked

TABLE 23.—ANALYSES OF GALL STONES (DRY WEIGHT)

| Case No. | Diabetes | Number of stones | Cholesterol per cent | Calcium per cent |
|---|---|---|---|---|
| 793 | − | 1 | 96.4 | |
| 4319 | + | | 0.15 | 34.0 |
| Ayers | + | | 93.2 | 1.3 |
| Clough | + | | 91.5 | 0.92 |

variation in chemical constitution that may occur among stones from diabetics. However, the frequent occurrence of cholesterol-rich stones in the diabetic series and the statistical evidence suggest a connection between diabetes, hypercholesterolemia, and gall stones.

Wilder[39] felt that the unusually high incidence of gall stones in diabetics previously reported by Root and Warren[41] could not be substantiated by the results in his series, although his figures were comparable to theirs, as he

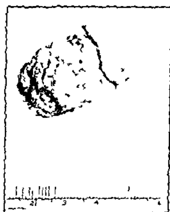


FIG. 53.—Large single cholesterol stone with small amount of calcium at one end found in nondiabetic patient. Female aged fifty-one years.

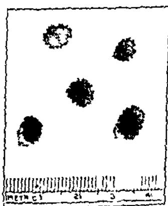


FIG. 54.—Calcium and pigment stones from female, aged sixty years. Duration of diabetes 10.7 years. This is one of the few cases of diabetes showing the calcium or pigment stone rather than the cholesterol type of stone.

considered the difference in prevalence between his diabetic and control series slight. However, it must be remembered that the control series which he used for comparison was based on material from the Mayo Clinic which would naturally be weighted with cases of gall-stone disease.

There is no one type of stone that occurs to the exclusion of others as the figures well show, although if any could be picked out as typical one may consider it the large ovoid stone made up of a mass of radiating cholesterol crystals with or without a small amount of calcium and pigment. To sum up, diabetics are known to have a disturbed fat metabolism and stones are more common in diabetic than in nondiabetic individuals.

## IV. THE LIVER

One of the oldest references to the pathology of diabetes is to the marked fatty infiltration of the liver that is sometimes present.* Mead[42] called attention to this in 1748. Adelsberg and Porges[43] consider an enlarged and fatty liver as a regular finding in diabetes.

Eppinger[44] states that the fat content of the normal liver is 1 to 2 per cent of its total weight, but that under pathological conditions the fat may increase to 40 per cent or more.

TABLE 24. WEIGHTS OF DIABETIC LIVERS

*Part A*

Liver Weights—Cases up to January 1, 1930

| Age, yrs | Total | Below normal | Normal | Above normal |
|---|---|---|---|---|
| 0 to 9 | 5 | 1 | 4 | 0 |
| 10 to 19 | 9 | 3 | 6 | 0 |
| 20 to 49 | 38 | 9 | 7 | 22 |
| 50+ | 111 | 28 | 45 | 38 |
| | 163 | 41 | 62 | 60 |

*Part B*

Liver Weights—Cases January 1, 1930 to September 1, 1937

| Age, yrs | Total | Below normal | Normal | Above normal |
|---|---|---|---|---|
| 0 to 9 | 0 | 0 | 0 | 0 |
| 10 to 19 | 6 | 0 | 3 | 3 |
| 20 to 49 | 35 | 3 | 15 | 17 |
| 50+ | 174 | 47 | 66 | 61 |
| | 215 | 50 | 84 | 81 |

*Part C*

Liver Weights—Cases September 1, 1937 to September 1, 1938

| Age, yrs | Total | Below normal | Normal | Above normal |
|---|---|---|---|---|
| 0 to 9 | 2 | 0 | 0 | 2 |
| 10 to 19 | 6 | 0 | 0 | 6 |
| 20 to 49 | 45 | 6 | 12 | 27 |
| 50+ | 271 | 67 | 113 | 91 |
| | 324 | 73 | 125 | 126 |

* Here again it is recognized that a fatty liver is not necessarily a sign of disturbed fat metabolism—it may well indicate injury of the cells due to some other cause.

In Table 24 the livers are grouped by weight and age. For weights under twelve years of age those of Coppoletta and Wolbach[45] are considered normal; for ages 13 to 19 a range of 900 to 1400 grams; for adults the range from 1400 to 1800 grams has been accepted as normal. The table is divided into three parts, the first made up of those cases dying prior to January 1, 1930, the pre-insulin cases and those of the early Banting Era, and the second and third parts of those cases dying since that date. Most of the latter received more liberal carbohydrate diets and more insulin. However, the liver weights are strikingly *similar in the three series. While there is a* slight trend toward heavier livers in the diabetic patients, this is far from striking.

*While it has been generally* assumed that the increase in size and weight of the liver in diabetes is due to fat, this does not exclude other factors. Enlargement of the liver has been shown to be due to glycogen storage, to circulatory changes, amyloidosis, and metastatic tumor, as well as fat. Of much importance is the fluid content of the liver. Best[46] has noted that the liver water content tends to diminish as the fat content increases.

Chiari[2] emphasizes the frequent occurrence of fat in the Kupffer cells in the liver, and holds that the amount may be related to the severity of the diabetes.

The more recent series of autopsies contains a high proportion of coronary deaths, hence passive congestion of the liver may be a factor.

*The abdominal pain not infrequently associated with* a large liver in the diabetic has been suggested by Marble[47] as secondary to stretching of the capsule. This suggests a rapid change in size—perhaps too rapid for fat deposition—but quite compatible with marked *increase in intracellular* fluid.

In a series of diabetic and nondiabetic livers analyzed in this laboratory by Dr. Nellie Halliday, the total fatty acid ranged from 2.2 to 4.3 *per cent* wet weight of fresh liver in the nondiabetic cases and from 4.1 to 10.8

formalin fixed liver.

The possibility of fatty degeneration of the liver in toxemias due to infections accompanying the diabetic state must be considered, but this might explain only a limited number of the cases.

As has been already mentioned, large amounts of fat in the liver do not preclude the presence of normal or even large amounts of glycogen. Fatty *infiltration may occur in* von Gierke's disease.[48]

The fatty livers found in experimental diabetes[49] are of much interest. Dragstedt's assumed insular hormone, lipocaic, is effective in preventing ... *in the livers* of depancreatized dogs when given in

Possibly the supply of this hypothetic hormone might be credited to the α or D cells the functions of which thus far are unknown. However in a few cases whose liver weights were above normal no demonstrable change was found in any of the insular cells.

Considerable question has been raised in the recent literature as to the existence of lipocaic and its possible identity with choline or other factors[51-61].

The effects obtained by feeding raw pancreas, lecithin[65], choline[66] or betaine hydrochloric acid[67] permit survival of depancreatized insulin treated animals without appreciable hepatic abnormality.

Large livers were noted by clinical examination in 12 of 44 juvenile diabetic patients and in only one of 231 diabetics over twenty years of age (Hanssen[59]).

Clinical evidence of the effect of lipocaic has been presented by Grayzel and Radwin[58] who found that regression of enlarged livers in three diabetic children was obtained with lipocaic and on discontinuance of its use enlargement reoccurred in one to two months. They also noted that the decrease in size of the liver was paralleled by a lower total serum lipid.

Marble[47] *et al* concluded choline is not a major factor in controlling hepatomegaly as the diets of the children with hepatomegaly they followed contained adequate choline and good response was obtained from the better regulation of their diabetes through protamine zinc insulin.

It is interesting that the hepatomegaly of galactose diabetes seems to be due to fat[60].

A useful discussion of the xanthomatous diseases including those involving the liver from the clinical and physiological standpoint has been presented by Thannhauser[27] and by McHenry and Patterson[58].

Probably the most extensive work to date on the liver is that of Zimmermann *et al*[61]. They biopsied the liver in 28 diabetics with uncomplicated well-controlled diabetes. Sixteen of these showed vacuolization of liver cell nuclei presumably representing glycogen. Fourteen patients showed fatty metamorphosis in varying degree, and in 3 of these cirrhosis was found. In 8 of the patients with fatty change there were significant amounts of iron-containing pigment in the liver cells. No relation could be established between the liver changes and previous control of the diabetes. They found only slight impairment of liver function tests in their patients.

In a further study[62] the same authors applied the glucose-insulin tolerance test of Himsworth to the same patients. They found that a positive correlation existed between the occurrence of fatty metamorphosis and insensitivity to insulin, also a positive but not conclusive correlation between fatty changes on the one hand and age, obesity and duration of diabetes on the other. They were unable to attribute the fatty change to nutritional deficiency, alcoholism or inadequacy of control of the diabetes. They speculate that pituitary hyperactivity might be responsible for both the fatty metamorphosis and the insensitivity to insulin.

Leevy, *et al*,[63] using a battery of liver function tests, found that 38 9 per cent of 380 diabetics had some evidence of liver dysfunction and demonstrated fatty change or cirrhosis in a few by biopsy However, Brown,[64] using 8 standard tests in 25 diabetics, found no evidence of impairment It is obvious that the selection of tests and the interpretation of results must account for some of this disparity

The claims of Taub, *et al*,[65] that the "juvenile' and adult' types of diabetes can be differentiated on the basis of liver dysfunction (which is held to be the fundamental cause of the adult type) have apparently not been confirmed by others

In any study of vacuolization and the like in liver cells it is important to keep in mind the wide normal variations The relation of diet to cytological changes in the liver is described in an interesting paper by Elman, *et al*[66]

## V THE ARTERIES

It is possible, though by no means proved that the increased incidence of atherosclerosis in diabetics may be associated with abnormal fat metabolism For further discussion of this question, reference may be made to Chapter 9

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## Chapter 7

# THE PATHOLOGY OF ACIDOSIS AND COMA

Acidosis and coma in diabetes are associated with the accumulation of the so-called *ketone bodies which are no longer regarded as abnormal metabolites* but are known to be formed normally and abundantly *in the course of fatty acid degradation They do not accumulate in the blood of normal* persons because they are normally consumed in *muscle and liver as rapidly as they are formed When glucose is scarce either because of lack of* dietary carbohydrate or because of loss of glucose in the urine or when glucose though abundantly present is not being catabolized at a normal rate *excessive quantities of fat are transported from the depots to the liver and* there degraded probably to two-carbon acetyl *fragments and a portion* of these are converted to acetoacetic acid *If these normal processes become* sufficiently exaggerated the *rate of acetoacetic acid formation will* exceed the body's capacity to destroy this compound and its level in the *blood will rise* The re-establishment of carbohydrate metabolism in such a subject appears to relieve the liver of the necessity of degrading fatty acids at excessive rates and as the *formation of ketone bodies resumes its* normal rate the ketone bodies which had accumulated in the body are either excreted in the urine or destroyed by the tissues of the body in their normal fashion (Stetten[1])

The pathological physiology of diabetic *coma appears to be exceedingly* complex especially as *regards electrolyte metabolism and it is only in* recent years that an insight has been gained into such factors as the all important role of potassium *Butler*[2] *in particular* has pointed out that many deaths have occurred after correction of the acidosis and after adjustment of the serum concentrations of sodium chloride *and glucose to* normal with no demonstrable cause of death being apparent at autopsy As he indicates such deaths may well have occurred in the past as a result *of therapy being focused on extracellular fluid* concentrations at the expense of intracellular needs particularly the need for potassium

The rapidity of the development of acidosis and its sequel coma depends not only on the rate and amount of production *of acid bodies but* also on the amount excreted and the rate of excretion which are closely *linked with the condition of the kidneys as well as the* amount of acid bodies taken up by the bases of the blood with displacement of the combined carbon dioxide

As diabetics have a striking tendency to develop *arteriosclerosis* renal arteriolar disease intercapillary *glomerulosclerosis* and pyelonephritis the kidneys are frequently *already impaired when needed to deal* with the main

tenance of acid base equilibrium Thus acidosis may be somewhat more likely to develop than in normal individuals even given an equal production of acetone bodies

The culmination of acidosis is coma The pathological changes in coma are conspicuous by their absence Even in pre-insulin days when diabetic coma developed in uncomplicated cases of diabetes there were no characteristic pathological changes that would enable one at post mortem or on looking at a section of tissue to say This material came from a case of diabetic coma Today the same holds true

Now the changes produced by acidosis of the acetone body type are so far as can be seen but little different from those seen in the acidosis so readily produced by intoxication of animals with various mineral acids as by the prolonged administration of hydrochloric acid Tubular damage due to toxic injury of the epithelial cells is a very frequent finding and still more impairs renal function thus furthering the development of a vicious circle This is the cause of the showers of casts in the urine so frequently associated with acidosis

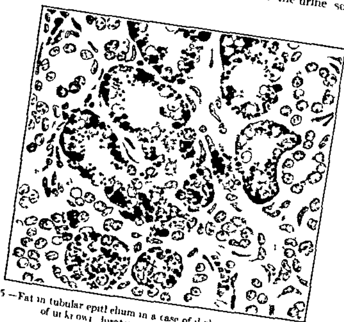


Fig 55 —Fat in tubular epithelium in a case of diabetes with sepsis Diabetes of unknown duration Sudan IV stain × 500

In cases of coma one sees at their best the various forms of pathological distribution of glycogen together with the absence of normal deposits which were discussed in Chapter [illegible] In fact some authors[3] have endeavored to explain the development of coma on the basis of glycogenic infiltration and vacuolization of the cortical cells Although we have not been able to

perform satisfactory glycogen stains on cerebral tissue from many cases of diabetic coma in the few cases that we have examined we cannot substantiate this theory

The antagonism between septic processes and insulin autogenous or injected is all too well known clinically A diabetic patient treated or untreated loses tolerance with even minor infections This is as true today as in the pre-insulin days When attacked by infection an insulin treated diabetic requires more insulin during the course of the intercurrent disease and promptly drops back to his former requirements when the infection is past The tables in Chapter 5 bring out vividly the fact that the one anatomically visible feature of carbohydrate metabolism glycogen storage tends to approach that of the normal individual in insulin treated cases but that in cases with sepsis the picture tends toward that of the untreated diabetic in spite of insulin Whether this is a direct antagonism it is impossible to say

Rare cases have been reported in which the liver showed normal or increased glycogen content after death from coma[4] Usually hepatic glycogen is depleted Popper and Wozasek[5] give liver glycogen values ranging from 75 mg to 1 9 mg in 8 cases of coma with most values toward the lower extreme As noted in Chapter 5 Bondy and Sheldon[6] found only a trace of glycogen in a liver biopsy from a case of coma but noted restoration of glycogen within a few hours of treatment

Let us consider the typical pathological findings in a case of acidosis progressing to death from coma in a non insulin treated diabetic The only changes which we find are those characteristic of prolonged acidosis—practically nothing but renal tubular changes sometimes with evidence of toxic injury to the liver cells

The kidneys are responsible for severe functional disturbances and some of the cases at autopsy show varying degrees of acute tubular damage often with marked fatty degeneration[7] of the tubular epithelium Anuria or oliguria is not infrequent[8,9]

Rarely renal insufficiency may be so marked as to prevent the excretion of the acetone bodies in coma so that ketonuria may not appear[10] Nitrogen retention may be very marked and showers of casts and albumin may be present in the urine

However the changes in the epithelium of the renal tubules in cases dying of coma are not comparable with the acute damage of toxic origin The necrosis is less marked the absence of polymorphonuclear leukocytic exudate is striking

The liver cells are practically devoid of glycogen their nuclei distended with it The epithelium of the kidney tubules shows large amounts that in the heart muscle is increased while in the striated muscle and skin it is depleted

The pancreas may show almost any type of lesion in the islands or in the acinar tissue or it may show none Fulminating cases of diabetes rapidly

progressing to coma and death frequently show hydropic change of the islands

One fairly frequent post mortem finding is the occurrence of blood fresh or partly digested in the stomach and intestinal tract Usually no bleeding point can be found and presumably capillary hemorrhages rather than gross bleeding points are the source At times the stomach in particular is tremendously dilated causing marked distress This distention may be a



FIG 56 —Extreme acute dilatation of the stomach in diabetic coma Photograph by courtesy of Prof Dr H Berning

of acute gastric dilatation in diabetic coma has been emphasized by Berning one of whose illustrations is reproduced in Figure 56

Lesions similar to those of acute asphyxia—capillary dilatation pericapillary edema degeneration of ganglion cells—have been reported by

Dillon, Riggs and Dyer[2] in the brains from 8 fatal cases of coma. This capillary dilatation may help to explain the frequent mucosal gastroenteritis.

the [illegible] in cases of coma [illegible] illustrates not too convincingly similar changes in a single case. We have not found these changes. Windle et al[15] found no definite cytologic changes in guinea pigs made severely acidotic by ammonium chloride given by stomach tube, by lactate buffer given intravenously, or by breathing 30 per cent carbon dioxide.

The eye changes in coma are discussed in Chapter 16.

A few of these coma cases were accompanied, as would be expected, by disturbed metabolism of the higher lipoid substances. Of the 11 cases of abnormal lipoid distribution mentioned in Chapter 6, 5 died in coma.

Lipemia in coma may be most striking; the blood may resemble melted strawberry ice cream, with a total lipid of 19.9 per cent. When studied by dark field microscope, such a blood shows myriads of droplets. Zon and Warren[16] studied the chylomicron count in a number of normal and diabetic patients, but found no relationship to cholesterolemia. However, the highest counts occurred in diabetics. (See also Chapter 9.)

The doughy consistency of the abdomen frequently noted in coma is due usually to two factors: distention of the colon by retained feces and distention of the stomach. Often dilatation of the bladder may be extreme.

Striking evidence that the anatomical changes in uncomplicated coma are reversible, and hence short of actual cell destruction, is given by the clinical course. Given a case of coma pure and simple, insulin treatment brings prompt recovery, far more rapid than would have been possible if any marked injury were present.

The development of insulin treatment has strikingly altered the picture in so far as deaths from coma are concerned.[7] This is well shown in the table in Appendix B.

Acute or subacute pancreatitis was associated with several of our cases. Four of these were under twenty years of age. Foord and Bowen[18] have reported 2 cases of acute pancreatitis in young diabetics dying with coma.

Little attention seems to have been given by clinicians to acute pancreatitis as a possible precipitating cause of coma in an established diabetic. Its occurrence is emphasized by Root.[19]

The skin of coma patients may be very susceptible to infection. Rivers[?] has described dermatitis gangrenosa occurring in coma.

Coma with or without infection may be accompanied by a high white cell count, which greatly complicates the picture from the clinical standpoint. A white cell count as high as 97,000 with frequent myelocytes has been reported[21] in coma without any evidence of infection, and the blood picture returned to normal and remained so after recovery from coma.

Recent studies on electrolyte changes in diabetic acidosis will doubtless

prove of great importance in the understanding of the condition although as yet they are without known pathological counterpart Holler was apparently the first to recognize the importance of potassium deficiency and his paper has been followed by many others confirming his observations[23 24] Martin and Wertman[25] studying 14 cases of severe diabetic acidosis found a marked fall in serum potassium in 4( per cent and a considerable drop in serum magnesium in 36 per cent The prime importance of the electrocardiogram in assaying the disturbance of potassium metabolism is indicated in Chapter 11 Franks *et al*[26] found that although the plasma inorganic phosphorus was elevated in all their cases prior to treatment a precipitous fall occurred in both the plasma level and urinary excretion of phosphorus when treatment was begun suggesting a depletion of stores of this element Butler[2] has recently written an able summary of the pathologic physiology and management of diabetic coma emphasizing particularly the importance of considering the intracellular as well as the extracellular electrolyte balance

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## Chapter 8

# DIABETES AND INFECTION

INFECTION was the cause of death in 213 of the 818 fatal cases of diabetes. It is a striking fact that the incidence of death from infection in the cases summarized in the last edition of this book was 38 per cent, while in the cases added since that time (1938) the incidence is only 6 per cent, a change doubtless attributable largely to the modern antibiotics. Tuberculosis and syphilis are omitted from the total number, only the acute infections being considered. Eighteen additional deaths were due to tuberculosis and 2 to syphilis. Moreover, a number of cases of gangrene are included, only those cases being considered where generalized sepsis or gas gangrene was the cause of death. Pneumonia was responsible for more deaths than any other localized infectious condition. There were 38 deaths from bronchial pneumonia, and 10 deaths from lobar pneumonia.

Just as arteriosclerosis is the bugbear of the diabetic who is doing well, so infection is the fear of the diabetic who is doing poorly. The more severe the diabetes, the more susceptible he is to infection. Once a diabetic is infected his diabetes becomes far more severe. As stated previously, sepsis is the greatest foe of insulin.

Whether or not we can translate to man the result of the experiment of Rosenthal and Behrendt[1] who found that fresh pus mixed with insulin abolished the insulin effect when it was injected into rabbits, nevertheless clinical observation has long shown that there is a marked increase in the severity of the disease during infection. As Joslin[2] states, "Insulin will not maintain a sugar free urine during an active infection unless given in very large doses." Moreover, when these large doses are given there is the constant fear that with sudden recovery an insulin reaction may result.

Inasmuch as many cases of the acute infectious diseases show central or focal necrosis of the liver or an acute renal tubular necrosis, it is reasonable to suppose that other parenchymatous organs, such as the pancreas, are acted on as well, particularly where the pancreas of the diabetic is just on the ragged edge, its islands barely producing enough insulin to keep the organism going and needing to be helped out with either dietary control or extraneous insulin. It is obvious that any very slight infectious process or toxemia may be sufficient to injure the island cells. Thus the already too delicate balance that is being maintained is overthrown.

We have seen this toxic injury to the islands in a number of instances, both in nondiabetic and diabetic patients. One of the most striking cases is that of a nondiabetic patient who died from lobar pneumonia. One of

us (S W)[1] had the opportunity of studying this case No 1C while in Dr Mallory's laboratory Most of the cells in the islands of Langerhans were necrotic or showed some evidence of injury There were as well numerous mitotic figures sometimes as high as seven in a section of a single island showing that there had been serious damage It is quite possible that cases such as these may give the clue to the transient glycosurias that occasionally appear in nondiabetic patients in the course of severe acute infections

As previously suggested it is perfectly reasonable to explain the severe drop in sugar tolerance noted in diabetic patients during acute infection in this same way The one hopeful feature is that the diabetes is not made permanently worse or rather the sugar tolerance is not kept at the same low level to which it is forced during the course of the infection The quick recovery of the diabetic patient after the infection has gone is very striking indeed suggesting that if the effect is on the insular epithelium it stops short of actual cell necrosis

It should be added that the evidence is by no means clear that the above mechanism is operative nor if it does indeed occur that it is the only one involved Other possibilities are listed by Marble[4] as follows (*See also* Greene and Keohen[5]) (1) increased production of hormonal antagonists (2) destruction of insulin by enzymes in leukocytes or pus (3) interference with the storage of glycogen None of these mechanisms can be regarded as being supported by anything like adequate evidence

Also Menkin[6 7] studying the mechanism of enhanced diabetes associated with inflammation in depancreatized dogs concluded that there was increased proteolysis at the site of inflammation and that the products of this increased protein catabolism seemed to lead to increased local gluconeogenesis from protein He also stated that insulin appeared to repress both the increased proteolysis and the gluconeogenesis This work requires confirmation

The types of infection vary but pneumonia and pyogenic infections stand out above all others

To protect the diabetic against pneumonia there is relatively little that we can do except to maintain his diabetes in as favorable a condition as possible If anything it is necessary to be overcareful with regard to colds and upper respiratory tract infections in the hope of checking them before pneumonia may have an opportunity to develop

After pneumonia the pyogenic infections are the most important particularly those of the staphylococcus and the streptococcus groups An intact skin is essential to a diabetic patient for it is his best safeguard against infection Blisters and scratches that would be insignificant to the *normal person must be carefully guarded from the access of bacteria* Even organisms which ordinarily are of very low virulence are capable of setting up severe infections Thus *Staphylococcus albus* is ordinarily considered of little significance and indeed if found in a blood culture is generall

dismissed as a contaminant yet it may be an extremely dangerous organism *to the diabetic* responsible for pyemia and multiple abscesses just as in certain cases in children it may be responsible for osteomyelitis

The diabetic skin is fertile soil for the pyogenic bacteria The carbuncle with its tendency to develop septicemia with the subsequent establishment of multiple abscesses is one of the most dangerous types of cutaneous infection Since these organisms are always present in the skin waiting for an

[illegible]

course of tremendous importance in both reducing the number of organisms present on the skin and in keeping in check their opportunities for entering wounds

Pyogenic infections caused 102 deaths In few cases aside from the fulminating osteomyelitis of children is there as widespread distribution of abscesses *as that found in some of these cases Fortunately thanks to the* modern antibiotics such cases are becoming rare

Except for their extent and tendency to become generalized furuncles and carbuncles in diabetic patients differ in no regard from those in non diabetics

Infection and gangrene are a particularly dangerous combination to the diabetic Infection is not only a frequent complication of gangrene due to he low resistance of the undernourished and necrotic tissue leading frequently to the establishment of generalized sepsis but it may be the under ying cause as well Even a low grade infection may be sufficient to overome the narrow factor of safety barely maintained in the diabetic leg ith arteriosclerosis of the diabetic type and result in the establishment *f the gangrenous process*

An odd finding secondary to high sugar content of the blood and tissues as encountered in a diabetic with pulmonary infection due to Aspergillus arge amounts of oxalates were excreted in the urine and numerous rystals of calcium oxalate were present in the sputum [8]

Three cases of mucormycosis of the central nervous system were reported y Gregory *et al* [9] in diabetics These cases were remarkable in that all resented the same triad (1) uncontrolled diabetes (2) evidence of orbital nfection and (3) meningoencephalitis associated with the presence of rge nonseptate hyphae An entirely similar case [illegible] addition was described by LeCompte and Meissner [10]

Excess sugar in the urine may lead to pneumaturia when a sugar fer*enting organism gains access to the bladder* [11] Air introduced by catheter ust be ruled out

Two very striking and unusual cases called attention to the clinical nportance of the high sugar content of the blood and tissues *in cases of* abetes with infection Both these cases had been clinically diagnosed as as *gangrene but repeated cultures* both aerobic and anaerobic as well as

guinea-pig inoculation failed to reveal anything other than colon bacilli and staphylococci In an additional case formation of gas by organisms in a perinephric abscess outlined the kidney strikingly in the roentgenogram

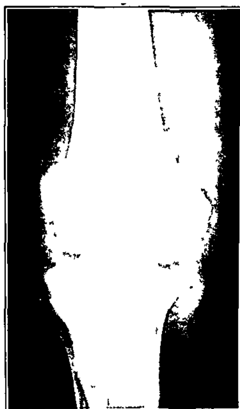


Fig 37 Roentgenogram of infected knee of diabetic patient showing gas bubbles in soft tissues around the joint

Case No 3747 was a man aged fifty-eight years whose diabetes was known to have existed for only a month although undoubtedly it had been present for some time previously He had suffered from intense pruritus and through scratching had produced several infected wounds in the inguinal regions The cause of death was an extensive phlegmonous abscess involving the inguinal region and the left flank At post-mortem as in examination during life there was marked crepitation of the tissues but a considerable amount of rather creamy pus together with a definitely purulent condition of the underlying muscles, coupled with repeated cultures negative for *Clostridium welchii* and positive for *Escherichia coli*, led us to

believe that the latter was the offending agent. Cultures and animal inoculations at post mortem also failed to produce any organism other than the colon bacillus. This strain of *Escherichia coli* was an extremely active gas producer. Unquestionably this organism in the sugar laden tissues had succeeded in producing the amount of gas that had proved so confusing from the clinical standpoint.

Focal infections are of considerable importance in keeping down sugar tolerance and are as frequent in diabetic as in nondiabetic patients. Teeth, tonsils and gall bladder are all possible points of localized chronic inflammatory processes. Here again the pathology is similar to that of nondiabetics.

Gingivitis is as frequent an accompaniment of diabetes as is caries. Kent[12] felt that periodontoclasia (pyorrhea alveolaris) was more common in the diabetic than the nondiabetic person. When diabetic children are brought under control progressive dental caries is arrested.[13]

The importance of infections of the urinary tract has been emphasized by Sharkey and Root,[14] who found 18 per cent of 196 diabetic autopsies to show purulent infection of various portions of the tract, and by Baldwin and Root.[15] The frequency of chronic and acute pyelonephritis is emphasized in Chapter 12. Prostatic abscess when clinically obvious is highly dangerous to the diabetic patient.

The reasons for the increased susceptibility of diabetics to infection are far from clear. Marble *et al.*[16] discussed the following as *possible* causes: (1) increased sugar content of blood and tissues; (2) decreased activity of blood elements associated with resistance to infection, such as (a) subnormal activity of complement, (b) subnormal phagocytizing capacity of leukocytes, (c) subnormal bacteriostatic and bactericidal action of whole blood; (3) inadequate functioning of fixed tissue cells; (4) lowered capacity of tissues to react to antigenic stimuli; (5) lowered state of general cellular nutrition.

Present-day opinion is opposed to the simple explanation that the increased sugar in blood and tissues promotes more rapid growth of organisms, since there is no valid evidence to support this point of view. As regards the other possibilities, the evidence is also indecisive. Marble *et al.*[16] found that fresh defibrinated blood and heparinized whole blood of diabetics had essentially the same phagocytic, bacteriostatic and bactericidal power against selected strains of streptococci as did blood from normal controls. The results in individual cases could not be correlated with the duration, severity, or state of control of the diabetes.

Richardson[17] concluded from testing the ability of diabetic and nondiabetic blood to kill *E. typhosa* that complement in diabetics does not differ from that in nondiabetics, but that the blood of diabetics had a slightly weaker bactericidal property and that the diabetic patients were less able than the nondiabetics to form agglutinins. However, the differences were not striking. Later[18] he found that underfed rabbits with a

depleted liver glycogen developed lower agglutinative titers against typhoid vaccine than did well fed controls with abundant liver glycogen. In further experiments the same author [9] found an apparently significant correlation in normal rabbits and depancreatized cats between the percentile amount of glycogen in the liver and the survival time after intravenous inoculation of bacteria. Likewise bacteria were found more frequently in the organs of depancreatized cats twenty four hours after intradermal inoculation of bacteria. However alterations in blood sugar, cholesterol, proteins and liver glycogen did not appear to influence this dissemination.

Recently Cruickshank and Payne [0] found that a Type II pneumococcus inoculated into whole blood of rabbits with alloxan diabetes and incubated *in vitro* grew much more rapidly than in normal blood. However when plasma alone was inoculated growth tended to be heavier in the normal plasma. They therefore suggest that there may be a defect in the leukocytic defense of animals with alloxan diabetes.

Flick *et al* [21] testing the antibody response of diabetic patients to typhoid immunization found a positive correlation between the antibody response and the serum albumin concentration.

## SYPHILIS

While syphilis particularly in the congenital form with its tendency to involvement of the liver and pancreas is a possible etiological factor in the production of diabetes, nevertheless the incidence of demonstrable luetic pathology is very low. We have not seen a pancreas in this series where it seemed that syphilis could be responsible for the changes present, and even the occurrence of syphilitic lesions in other organs is very rare. Warthin and Wilson[22] emphasized the importance of syphilitic pancreatitis but our material is apparently very different from theirs.

In our series 2 deaths only were due to the disease. One case, a colored female aged fifty years with diabetes of three years duration died of hemorrhage from a syphilitic ulcer of the trachea. The other case, a male aged forty two years, died from a ruptured abdominal aneurysm. His diabetes had been present for three months.

## TUBERCULOSIS

Although tuberculosis was formerly responsible for many of the deaths of diabetic patients only 18 deaths from tuberculosis occurred in this series. This is probably abnormally low as cases with advanced pulmonary tuberculosis are often discharged to their homes or to sanatoria.

This is in marked contrast to a generation ago when nearly half the fatal cases of diabetes died from tuberculosis. However the decrease in rate of incidence of tuberculosis in adult diabetics is not as rapid as the decrease of tuberculosis in the general population [3]

The course of tuberculosis complicated by diabetes seems to be in no way remarkable as long as the diabetes is properly controlled[24-26] A curious observation in regard to the pathology is that of Wiener and Kavee[27] apparently confirmed by Root and Bloor[28] that dense pleural adhesions seem to be only about half as frequent in diabetics as in nondiabetics Apparently this is correlated with clinical recognition of greater ease of inducing pneumothorax in diabetics with tuberculosis However, since diabetes is not known to influence granulation tissue or fibrosis one wonders whether this finding may not indicate a selection of less slowly progressing cases (one thinks of pleural adhesions as associated with tuberculosis of fairly long duration)

For extended information on tuberculosis in diabetes reference may be made to the chapter by Root in Joslin s book[25]

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# Chapter 9

## VASCULAR DISEASE IN DIABETES

DURING the last few years a shift in emphasis has occurred with regard to diseases of the blood vessels in diabetes. While sclerosis of the larger arteries, especially the leg vessels and the coronary arteries, is still recognized as a major problem, careful observation of diabetics who have survived ten, fifteen, twenty or more years with their disease makes it increasingly clear that the disabilities in this group are largely referable to disorders of the smaller blood vessels (i.e., arterioles, venules, capillaries).[1–6] The lesions affecting these small vessels seem to be fundamentally different from those involving the larger elastic and muscular arteries, and in many respects they seem to be characteristic of, if indeed not specific for, diabetes.* Since, however, the effects of involvement of the small vessels are usually manifest in either the kidney or the eye, discussion of this subject will be found in the chapters devoted to these organs and to the cases of long duration (Chapters 12, 13, 19). The present chapter will be concerned with disease of the larger arteries, i.e., atherosclerosis and medial calcification (Monckeberg's sclerosis).

### ARTERIOSCLEROSIS OF THE LARGER VESSELS

Whether the increased prevalence of arteriosclerosis in diabetes is due to the disease itself or to methods of treatment of the disease, the importance of the problem is such that it cannot be disregarded. In the first edition of this book the statement was made: "I have yet to see at autopsy a diabetic or to read the autopsy protocol of a diabetic whose disease has lasted five years or more, free from arteriosclerosis, regardless of age." Since then a few cases with duration over five years have been found to be practically free from arteriosclerosis, but they are not many.

Of course, many of our cases fall in the older age group and therefore would be expected to have arteriosclerosis in any event. However, the cases in the younger age groups frequently show this lesion, as has been pointed out in Chapter 6 in considering the pathological evidences of abnormal fat metabolism. If we accept tentatively Aschoff's[7] modification of Virchow's imbibition theory of arteriosclerosis, which in essence seems to

* It is unfortunate that the word "arteriosclerosis" is used so loosely to include the retinal and renal lesions of diabetes, especially in the young patient. The characteristic microaneurysms and hyaline capillary glomerular changes seem to be, in fact, diseases of capillaries.

be still the most popular theory of pathogenesis of the disease it is obvious that blood plasma laden with lipoid will predispose to the development of arteriosclerosis and the lesion may be correlated with abnormal metabolism

In the days when excessively large amounts of fat were given to diabetic patients a direct cause and effect relationship between the ingested lipoids and the fatty plaques of the aorta and other large arteries seemed so clear that little question was raised concerning it Today however after almost thirty years of insulin and relatively low fat diets we are still faced with a disturbingly high incidence of coronary occlusion in diabetics and atherosclerosis accounts for a much higher percentage of deaths among diabetics than among nondiabetics

In Chapter 19 it is shown that fully 43 per cent of a series of 157 patients who had had their disease for over 15 years died as a direct result of atherosclerosis of the coronary arteries Most of the rest of course had atherosclerosis although it was not a direct cause of death This figure is to be contrasted with a study of a large general autopsy series (predominantly nondiabetic) in which only 4 per cent of deaths were directly attributable to disease of the coronary arteries (see Chapter 11) Also as shown strikingly in Appendix B deaths attributable to arteriosclerosis rose from a mere 17 5 per cent in the early Naunyn era of diabetes to the staggering figure of 69 4 per cent in recent years (1944 to 1949) In other words atherosclerosis emerges without question as the leading cause of death in diabetes accounting for over two thirds of the fatalities

Atherosclerosis is such an important cause of death in nondiabetics also that a tremendous amount of effort is being devoted especially in the more recent literature to attempts to elucidate the mechanism by which the characteristic intimal plaques are produced The history of the disease has passed through many phases and as will be noted below the present phase is dominated by the study of cholesterol and the lipoproteins It is because of the general importance of the disease and because there seems to be no difference in kind in the atherosclerosis of diabetics and nondiabetics that much of the discussion in this chapter contains no direct reference to diabetes

Atherosclerosis is most easily studied at the autopsy table but it is obviously of great importance to develop clinical methods for detecting its presence activity progression or regression in the patient before death

In the examination of the living patient there are numerous ways in which the presence of arteriosclerosis may be detected The method of palpation of the arteries is old one is almost tempted to say as old as it is uncertain The examination of ocular fundi gives an excellent index of arterial disease in many cases but no one has yet shown how close the correlation between retinal and systemic vascular disease may be

Morrison and Bogan[3] have shown the great help that the roentgen ray may give in the diagnosis of arteriosclerosis with calcification.

However, this diagnostic method can take no cognizance of atheromatous or other changes even though they may be very extensive, since it records the deposition of calcium salts.*

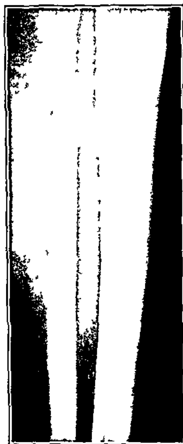


Fig. 58. Roentgenogram of leg of 21-year-old diabetic man showing calcified artery.

By these methods White[3] found evidence of arteriosclerosis in 24 of 104 diabetic children; 19 showed calcification of leg arteries and 5 showed retinal sclerosis alone. Since her earlier report she has used the roentgen ray effectively in gauging the presence of vascular disease not only in children but also in pregnant diabetic women (Figs. 58 and 59).

* Calcification of the vas deferens is also common in diabetes. Its relation, if any, to arterial disease is obscure.

Recently Lindbom[10] has published a beautiful demonstration of the value of refined arteriographic techniques in the study of vascular disease in the legs. An extensive earlier study was that of Ruhl.[1]

Our other means of diagnosis are clinical: the physical signs and symptoms of local circulatory deficiencies. Some of these are of definite value, such as the pain in claudication, the occurrence of gangrene, color changes, the presence or absence of pulsation in the vessels and local temperature. Goodman *et al.*[1] in a *critical survey of methods of assaying vascular disease* in diabetics found the following techniques of greatest value: (1) carotid sinus pressure test,* (2) palpation of supraclavicular pulsations,



Fig. 59.—Calcified pelvic arteries in a 21-year-old diabetic woman.

(3) presence of calcification of the abdominal aorta and in the arch of the aorta, (4) determination of occlusive changes in the legs by palpation.

It must be emphasized that all methods for the diagnosis of generalized arteriosclerosis in the living patient are subject to gross error.

There are two main types of arteries: the elastic arteries, such as the aorta, the iliac and carotid arteries, and the muscular arteries, such as the *posterior tibial or brachial*, in which the development of the musculature very often overshadows that of the elastic laminæ. This muscular type is encountered chiefly in the peripheral and the splanchnic arteries. The arterioles also are of the muscular type.

* Th[illegible] on [illegible] auth[illegible] has the alleged value of the carotid sinus test in [illegible] arteriosclerosis [illegible]

is some doubt about this *mechanism* These calcium soaps become the *calcareous or even bony* plaques formed in the atheromatous regions As the plaque becomes larger and reaches nearer the surface the *blood stream may sweep away portions* of the pultaceous material forming the familiar *atheromatous ulcers*

As will be noted further below Lansing Blumenthal and co-workers have maintained in a series of papers[15-18] that *atherosclerosis is actually* closely associated with the process of aging and that the formation of atheromatous plaques is always preceded by calcification ( elasto-calcinosis ) of the media



Fig 61 —Femoral artery showing medial calcification or the Mönckeberg type of sclerosis The dark mass filling lumen of the artery is a thrombus

The second great type of arterial change is that seen in the muscular vessel Here medial calcification the Monckeberg type of *sclerosis* predominates This lesion is entirely distinct from the atheromatous process which we meet in the elastic vessels and is restricted to the muscular vessels Intimal change is negligible and the lumen *remains* intact or is even actually widened The calcium becomes *deposited in fine granules in* the connective tissue stroma about the muscle cells This calcification gradually becomes more and more abundant finally surrounding and causing atrophy of the smooth muscle cells It is sometimes difficult *to tell* whether the particles of calcium salts are outside or *within the muscle cells* which may be necrotic Any leukocytic reaction is extremely rare Not

infrequently organization of the calcified mass may take place with resultant formation of bone which may even contain marrow in the more advanced cases Through secondary intimal injury thrombosis may occur as shown in Figure 61 This is the type which is demonstrated in the roentgenogram

There seems to be little, if any, relationship between the occurrence of this type of arteriosclerosis and the atheromatous although occasionally the two forms may coexist (As noted above, one group of investigators[15, 18] holds that there is a close and important relation between calcification in the media and atheromatous plaques ) In the smaller arteries there may be

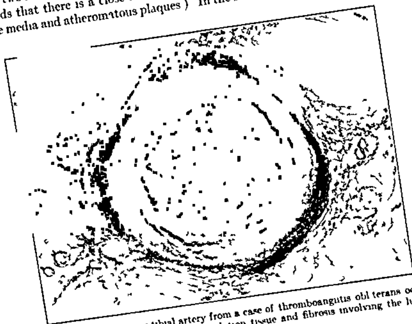


Fig 62 The anterior tibial artery from a case of thromboangiitis obliterans occurring in a nondiabetic Note the granulation tissue and fibrosis involving the lumen with some recanalization

thickening of the intima with or without the deposit of lipoids When this intimal change is at all marked one is justified in considering the possibility that the patient from whom the vessel was removed may be diabetic

The third great type of arterial change is that known as thromboangiitis obliterans or Buerger's[40] disease This type usually occurs in young adults Here thrombosis organization of the clot, and subsequent partial recanalization of the thrombosed vessels are the outstanding features followed by shrinkage of the walls and resultant reduplication of the elastic laminae Calcification is absent as is deposition of lipoids There is usually a very sharp distinction between the fairly normal-appearing adventitia and muscularis and the obliterated fibrosed lumen An excell … example is shown



*Fig* 63.—Arteriosclerosis involving the anterior tibial artery in a diabetic. Note the marked intimal thickening. There is also a considerable degree of medial calcification. Hematoxylin-eosin stain. × 10

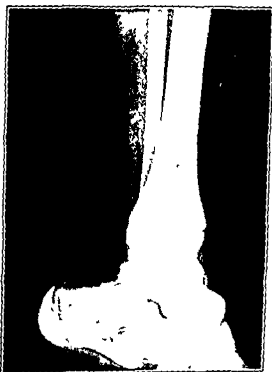


Fig 64.—Roentgenogram of the left leg of the diabetic boy whose legs were illus- [illegible] In the present picture the boy is twenty-one [illegible] ere is marked calcification of [illegible] ssels. Note the *failure of the* [illegible] wed dwarfism in addition to

in Figure 62. It is apparently not more frequent in the diabetic than in the nondiabetic.

In the diabetic individuals the atheromatous type of lesion is the outstanding one. The others may be present, but if there is one thing that should lead us to suspect that an artery in a given case comes from a diabetic patient, it is when we find the intimal type of involvement in the muscular arteries, either alone or superimposed upon the medial calcification.

In a series of 810 diabetic autopsies suitable for study of vascular lesions, 288 deaths (35 per cent) were due to arteriosclerosis or tissue changes immediately secondary to that lesion. Since 124 cases of the series were



Fig. 63.—Masses of cholesterol crystals from thickened intima of anterior tibial artery. Male, aged sixty-one years. Duration of diabetes unknown. Intimal change is accompanied by a moderate amount of medial calcification. [illegible] stain. × 375.

under forty years of age, this high prevalence of arteriosclerosis as a cause of death is even more striking. In the 300 cases of the first series, 72 (24 per cent) died from arteriosclerotic lesions. This slight relative increase is due probably to the sharp drop in deaths from coma. The percentage in cases of long duration is practically unchanged. Of the 157 cases of diabetes of fifteen years' duration or over, 94, or 60 per cent, died from arteriosclerotic lesions.

Of interest is Case No. 3296, a female, aged fifty years, who showed almost no arteriosclerosis although her diabetes had been present for fifteen years.

The heart led all the other organs with 201 of the arteriosclerotic deaths attributable to it. Of course, this is only a small proportion of the hearts showing the evidences of arteriosclerotic injury, as reference to Chapter 11 will show.

TABLE 25.—INCIDENCE OF ARTERIOSCLEROSIS BY DECADES

| *Age at death, yrs.* | *Total cases* | *Total with arteriosclerosis* | *Deaths due to arteriosclerosis* | *Arteriosclerosis absent* |
|---|---|---|---|---|
| 0 to 10 | 11 | | | 11 |
| 11 to 20 | 35 | 17 | | 18 |
| 21 to 30 | 40 | 30 | 4 | 10 |
| 31 to 40 | 38 | 34 | 5 | 4 |
| 41 to 50 | 88 | 82 | 14 | 6 |
| 51 to 60 | 197 | 195 | 65 | 2 |
| 61 to 70 | 257 | 255 | 129 | 2 |
| 71 to 80 | 132 | 132 | 66 | |
| 81+ | 14 | 14 | 5 | |
| ? | 4 | 3 | | 1 |
| | 816 | 762 | 288 | 54 |

TABLE 26.—ARTERIOSCLEROTIC DEATHS AMONG 816 DIABETIC AUTOPSIES

| *Arteriosclerosis most marked in* | *Number* | *Per cent* |
|---|---|---|
| Heart | 201 | 69.8 |
| Extremities | 2 | 9.4 |
| Brain | 28 | 9.7 |
| Kidneys | 17 | 5.9 |
| Not localized | 15 | 5.2 |
| | 288 | 100.0 |

TABLE 27.—ARTERIOSCLEROTIC DEATHS AMONG 157 DIABETIC AUTOPSIES, DURATION OF DIABETES FIFTEEN YEARS OR OVER

| *Arteriosclerosis most marked in* | *Number* | *Per cent* |
|---|---|---|
| Heart | 74 | 78.7 |
| Extremities | 3 | 3.2 |
| Brain | 10 | 10.6 |
| Kidneys | 6 | 6.4 |
| Generalized | 1 | 1.1 |
| | 94 | 100.0 |

Arteriosclerosis of the extremities as exemplified by fatal gangrene accounted for 27 deaths. The percentage of deaths from gangrene has been reduced to one-fourth of that in the first series. This reflects the value of educating the diabetic patient in the care of his feet, also the influence of the antibiotics.

Cerebral manifestations of arteriosclerosis were responsible for only 28 deaths, and vascular disease of the kidneys, although present in many cases, was fatal in only 17.*

This marked prevalence of cardiac and peripheral arterial involvement in diabetic patients contrasts strongly with the frequency of cerebral and renal damage in nondiabetics. That this is no peculiarity of the present series is shown by Table 28 giving the site of fatal arteriosclerosis in 4,495 cases.

TABLE 28. LOCALIZATION OF TERMINAL LESION IN 4,495 DIABETIC DEATHS DUE TO ARTERIOSCLEROSIS (JOSLIN[20])

| | Per cent of all deaths | | | | | |
|---|---|---|---|---|---|---|
| *Site of terminal lesion* | *Naunyn era* | *Allen era* | *Early Banting era* | *Late Banting era* | *Hagedorn era* | *Charles H. Best era* |
| Brain | 18 | 22 | 20 | 18 | 22 | 22 |
| Kidneys | 18 | 15 | [illegible] | 9 | 6 | [illegible] |
| Heart | [illegible] | 40 | [illegible] | 57 | 60 | 64 |
| Peripheral gangrene | 21 | 17 | 11 | 13 | 8 | 4 |
| Unassigned site | [illegible] | 6 | 3 | 4 | 4 | 3 |
| Number of deaths | [illegible] | 203 | [illegible] | 1474 | [illegible] | [illegible] |

Cerebral arteriosclerosis is no more frequently a cause of death in diabetic patients than in nondiabetic, in spite of the greater prevalence of arterial disease in the former group.

Taking the aorta as an index of arteriosclerosis, we find only 66 free from arteriosclerosis among the 783 which are recorded. Of these 158 showed slight involvement, 194 moderate, and 365 marked involvement. This tabulation includes cases of all ages, though naturally most lie in the older age groups.

## ARTERIOLAR SCLEROSIS

Disease of the arterioles usually takes the form of hyaline thickening of the intima, *i.e.*, the change so often associated with hypertension in the nondiabetic and described in the classical paper of Moritz and Oldt.[21] Sclerosis of the arterioles in the diabetic causes its most obvious damage in the kidneys. Discussion of this type of vascular change is therefore given in greater detail in Chapter 12.

## DISEASE OF THE CAPILLARIES AND VENULES

As indicated at the beginning of this chapter, disease of the smallest blood vessels has now come to occupy the center of the stage in discussion of the vascular complications of diabetes. For consideration of these lesions the reader is referred to Chapters 12, 13, and 19.

* In the group of young diabetics of long duration, renal disease is a major cause of death (see Chapters 12 and 18).

## ETIOLOGY OF ATHEROSCLEROSIS

No part of the whole field of pathology has been so much the domain of dogmatic statement as arteriosclerosis. Each generation in its turn has accepted some doctrine put forth with authority on the most insecure basis in fact and even now, as in some *religious discussions, there are those who* are insistent upon some explanation quite at variance with that equally vigorously maintained by another group.

The above statement by a distinguished student of the disease[22] appeared in 1933 and is still applicable today. Only the flimsiest sort of evidence is available as to the causes of *arteriosclerosis and one can do* little more than list various hypotheses.

In this section discussion will be limited to the intimal type of disease, atherosclerosis.* As indicated above this is the most important type of vascular disease, since it now appears to be the leading cause of death not only in diabetics, but in the *rest of the population as well. No considera*tion will be given to theories unsupported by any valid scientific evidence, such as those which hold the disease to be due to aging alone, tobacco, alcohol, syphilis, hypotension, lead, epinephrine, *etc.* Some idea of the tremendous amount of literature, both good and bad, which has appeared on the subject may be gained from the voluminous review by Hueper.[23] Other valuable recent reviews are those by Duff and McMillan,[24] by Katz and Dauber,[25] and by Gubner and Ungerleider,[26] to which the reader is referred for a more *detailed* discussion with numerous references.

The most obvious and characteristic feature of atherosclerosis is the *presence of lipids in both early and late lesions. Most theories regarding* the etiology and pathogenesis of the disease have therefore included some attempt to explain how the lipids get into the lesions and whether their presence *is indicative of a primary metabolic disturbance or simply rep*resents a secondary deposit in injured tissue.

One reason for the large number of theories concerning the pathogenesis of atherosclerosis is to be found in disagreement as to what constitutes the earliest lesion of the disease. Indeed, it is impossible to be certain of this *point, since in examining the tissues at necropsy one* is attempting to reconstruct a dynamic process from morphological evidence. It is usually necessary to assume, therefore, that the smallest lesions are the earliest, and it follows that such lesions will be found only by chance during the examination of many microscopic sections. Hence it is held variously that the earliest change is a swelling or necrosis of the intercellular ground substance,[7, 27] a fine deposit of extracellular lipoid droplets,[28] or a small collection of lipoid phagocytes.[14, 25]

The oldest and perhaps most widely *held theory of the pathogenesis* of atherosclerosis is the *imbibition* or *infiltration* theory. First enunciated by

* The medial calcification of Mönckeberg as seen in the diabetic does not differ from the same type of lesion in the nondiabetic. The pathogenesis is quite obscure.

Virchow, and popularized by Aschoff,[3] the theory holds that local loosening of the intercellular cement substance due to mechanical stress is followed by imbibition of lipid-containing fluid from the blood plasma into the vessel wall. Phagocytosis of the lipids by histiocytes occurs, followed gradually by fibrosis and growth in size to form the fully developed plaque. Aschoff distinguished an atheromatosis of youth in which the lipid infiltration was reversible, but more recent authors seem to agree that this process does not differ in kind from that seen in adults.

A variant of this concept is the "anoxemia theory" of Hueper,[illegible] based on experiments in animals with various macromolecular substances and holding that due to "an imbalance of plasmatic colloidal equilibrium" a thin film of cholesterol is deposited on the endothelium, leading to interference with the nutrition of endothelial cells, which become more permeable and are at the same time stimulated to take up cholesterol and become foam cells. Foam cells are also thought to be formed from subendothelial phagocytes.

In criticism of Hueper's theory it may be said that it is based on experimental work in which massive doses of macromolecular colloids (polyvinyl alcohol, methyl cellulose, pectin, and acacia) were injected intravenously into animals. As noted by Leary,[14] these substances are essentially foreign bodies which in large doses overwhelm the experimental animal and lead to its death; also the intravenous route is not a natural one. Further, the carry-over to the human situation has the defect of being a reasoning by analogy with insufficient supporting evidence.

The major role given to cholesterol in this theory is in accord with the modern trend which reflects the influence of the rabbit and its response to [illegible]

[illegible]

in the human disease is indicated by the work of Hirsch and Weinhouse,[illegible] who found the lipids of atheromatous plaques to be present in the same proportion as in the plasma, suggesting an infiltration from the blood rather than a selective absorption of cholesterol. Buck and Rossiter,[illegible] however, found differences in concentration of the various lipids which suggested to them that simple imbibition was not an adequate explanation.

[illegible]

hold that diabetes is without influence on atherosclerosis of the aorta and that the determining factors are age and blood pressure. However, only 4 of their 32 diabetics could be classified as nonhypertensive, and one wonders about the significance of this small number.

Another point of view was expressed by Winternitz *et al.*,[illegible] according to which most of the changes in atherosclerosis are regarded as mediated by the vasa vasorum, which can be demonstrated in abundance in and around

atheromatous plaques According to this theory, the lipids and other constituents of the plaque may be derived from exudation and hemorrhage *from the vasa vasorum Although such processes certainly are factors in the* life history of the older plaques, and in the formation of thrombi, they can hardly be regarded as effective in initiating the earliest lesions, since vessels are rare in the intima of the normal human artery. Also, it has been pointed out that the veins of humans, which are highly vascularized, and the arteries of some animals showing *extensive vascularity are only rarely the seat of atherosclerosis* [34]

Another theory of the origin of the lipids of the atheromatous plaque is *that of Leary,*[11] *who holds that invasion of the arterial wall by foam cells is* the origin of the plaque This point of view is based on years of experience in feeding cholesterol to rabbits The sequence of events *in the rabbit,* according to Leary, is as follows *when cholesterol is fed, there occurs a* latent period during which cholesterol esters are precipitated in crystalline form in the liver and are then taken up by the Kupffer cells, *which eventually escape from the sinusoids, traverse the lungs, and selectively invade* (by "chemotaxis") the intima of arteries from the flowing blood The lesions so produced are held to be entirely similar to the lesions of human *coronary atherosclerosis, although this point has been contested* [27] *Leary also contends that the earliest stages of the lesion, i e, invasion of the endo-*

the lipophages, by reason of their low specific gravity, would be expected to move toward the slowly-moving peripheral part of the blood stream, adjacent to the endothelium This type of *reasoning* is, of course, highly speculative

Leary has much evidence to support his conception of the sequence events in the rabbit However, the theory as applied to the human is seriously weakened by failure to demonstrate the part of the cycle involv-
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earliest lesion in both rabbit and man may be considered debatable *In* short, although Leary's hypothesis has much to recommend it, *the evidence for it can hardly be regarded as complete*

**The Rôle of Cholesterol** —It would be difficult to overestimate the importance of the rabbit in the history of thinking on the subject of atherosclerosis. The regularity with which this animal develops vascular lesions when fed cholesterol, as shown by Anitschkow[23] and the Russian school and later by Leary,[16] has doubtless been responsible more than any other factor for the current emphasis on cholesterol. In general other animals have been quite resistant to cholesterol induced atherosclerosis, but recently the chicken has been found to be a susceptible animal[3, 38] and also the dog (provided that thiouracil be given at the same time).[39]

This extensive experimental work in animals has led in the last few years to an extraordinary and probably unwarranted emphasis on the role of cholesterol, as distinct from other lipids, in human atherosclerosis. As will be seen below, most papers have reported only total serum cholesterol without reference to other lipids and often without reference to genetic and other factors which may be of major importance. The recent admirable papers of Ancel Keys[4, 41, 42] indicate the necessity of controlling some of the many variable factors before any valid conclusions can be drawn.

In general it may be said that efforts have been devoted toward answering two questions: (*a*) Is the level of serum cholesterol related to the degree of atherosclerosis in a given individual? and (*b*) Is the level of serum cholesterol related to the dietary intake of this substance?

As regards the first question, both negative and positive evidence is available. Lande and Sperry[43] studied the serum cholesterol in relation to the degree of atherosclerosis (measured by lipid content) in the aortas of 123 healthy persons who died by violence; they found no correlation. Ungerleider and Gubner[44] found no significant difference as regards cardiovascular disease between two large groups of insurance applicants, one having blood cholesterol less than 175 mg. per cent, the other over 200 mg. per cent. In another report,[45] however, they found distinctly less vascular disease in a group with unusually low blood cholesterol and raised the question as to whether the so-called normal range of blood cholesterol may not be unduly high. Several other recent reports have suggested a positive correlation between the height of the serum cholesterol and the presence of coronary sclerosis. For instance, Steiner and Domanski[46] followed 15 patients with coronary disease and 15 controls of comparable ages for periods up to two years and found an average blood cholesterol of 355 mg. per cent with a range of 308 to 490 in the coronary group and an average of 255 with a range of 214 to 334 in the controls. Of considerable interest is the work of Boas *et al*.[48] and Adlersberg *et al*.[4] who have demonstrated a striking familial incidence of high serum cholesterol, xanthoma, xanthelasma, arcus of the cornea, and coronary disease in certain families. They even suggest that xanthoma and often coronary disease develops in patients who carry two abnormal genes for cholesterol, *i.e.*, homozygotes, whereas in those with only one abnormal gene (heterozygotes) the only

manifestation of disturbed lipid metabolism may be an elevated serum cholesterol Wilkinson[48,49] has made similar observations

Although there does appear to be some evidence suggesting a relation between hypercholesterolemia and atherosclerosis the relation between serum cholesterol and the dietary intake of this substance is by no means clear Heymann and Rack[6] found no rise in serum cholesterol after giving large doses to children Steiner[51] likewise obtained negative results Shaffer[52] found no increase in coronary disease in a group of patients on a high cholesterol diet Keys[40,41,42] whose papers stand as models of careful investigation in this field found no correlation between dietary intake and serum cholesterol (except with extraordinary diets such as rice and fruit)[53] Keys also points out in a convincing way some of the many factors which must be taken into account before one can say that a given individual does or does not have a normal serum cholesterol Apparently a highly refined fat free diet (protein hydrolysate and dextrimaltose) will reduce serum cholesterol[3] but the practical significance of such observations is open to question Geographical evidence is unconvincing paradoxically both the Eskimos and the Chinese are cited as having a low incidence of arteriosclerosis the former being on a meat diet which must be fairly high in cholesterol the latter on a vegetable diet presumably low in cholesterol Gertler *et al*[55] point out some of the fallacies of this type of evidence Thannhauser[56] also emphasizes the weaknesses in the argument and holds that the vascular xanthoma of familial hypercholesterolemia is a different disease from arteriosclerosis In general it may be said that in man evidence for a relation between the amount of cholesterol ingested and either the height of the blood cholesterol or the incidence of atherosclerosis is not convincing[40,41,42,49,55,56] This seems to be true even though a fairly impressive case may be made out by citing only the positive evidence and neglecting the negative[57] At any rate attempts to prescribe low-cholesterol diets[58] to prevent arteriosclerosis would appear to be premature Best[59] dismisses the use of choline in arteriosclerosis as having no validity

In this connection the work of Wilens[60] is of interest He points out that overweight is common in all the important conditions that are associated with atherosclerosis and demonstrates on autopsy material a general correlation between obesity and the incidence of atheromatosis However Faber and Lund[61] appear to find no definite effect of obesity when hypertension is taken into account

One gets the impression that too much attention has been given to cholesterol alone without considering its relation to other substances particularly lipids in its absorption and metabolism especially as regards its stability in the blood stream As noted above it has been shown that the lipids of the atheromatous lesion are present in the same proportions as in the blood plasma They do not consist solely of cholesterol The papers of Gubner and Ungerleider[62] and of Gertler *et al*[55] and of Duff and McMillan[21] are especially valuable in pointing out the multiplicity of factors

involved.* The importance of fat in the intestinal absorption of cholesterol and the rôle played by phospholipids and proteins in maintaining the colloidal stability of cholesterol in the circulating blood are of great importance. It has been suggested that the ratio of phospholipid to cholesterol may be more important than the total cholesterol.[62,63] Kellner et al[64] showed that in rabbits injection of detergents resulted in a higher blood cholesterol but less arteriosclerosis; they found that the phospholipids were elevated along with the cholesterol. Simms et al[65,66] have described the presence of substances in plasma called "lipfanogens" which promote the formation of intracellular fat granules in tissue culture and also produce fat deposition in segments of adult chicken arteries in vitro. The action of these substances is said to be opposed by "antilipfanogen."

Also of much interest is the recently reported work of Becker et al[67] who have studied the chylomicron count in the blood after giving a fat meal (0.5 gm. oleomargarine per kg. of body weight). They found that the chylomicron count of young subjects reached a peak at two and one half to three hours and returned to the fasting level by the end of the fifth hour. In older subjects the count did not reach its peak for eight to twelve hours and did not return to the fasting level for about twenty-four hours. These results suggest that older persons have a continuous elevation of the chylomicron count and the authors suggest a possible relation to the occurrence of atherosclerosis.

Zon and Warren[68] found higher chylomicron counts in diabetics than in normals but could establish no relation to the cholesterol level of the blood. Moreton[69] has also made extensive studies of chylomicrons.

The new work of Gofman and associates[70] has attracted a good deal of attention. They characterized the larger molecules of the serum by ultracentrifugation, recording their results in terms of the flotation rate in Svedberg units ($S_f$). First studying rabbits they found that normally the serum contained a good many lipoprotein molecules of $S_f$ 5 to 8, with a cholesterol content of about 30 per cent by weight. In cholesterol feeding this lipoprotein increased, but there also appeared giant molecules of $S_f$ 10 to 30. A study of 104 patients with proved myocardial infarction revealed an almost universal occurrence of cholesterol-bearing molecules of $S_f$ 10 to 20 at fairly high levels. All their normal controls showed a lower frequency of occurrence of measurable concentrations of this class of molecule than did the myocardial infarction group. They also found significantly higher numbers of these molecules in males from twenty to forty years of age than in females of the same age group. In the age groups over forty both males and females showed significant increases in these molecules. Their data also seemed to indicate a "probably higher incidence" of measurable concentrations of molecules of the $S_f$ 10 to 20 class in a group of diabetics.

* A detailed recent review of the relation of lipids and lipoproteins to the pathogenesis of atherosclerosis is to be found in a series of articles in the American Journal of Medicine.[60]

On the other hand Keys [70a] holds that there is no evidence that the estimation of these giant molecules has any special virtue beyond that of simple cholesterol measurements in predicting atherosclerosis or in estimating the activity of the arteriosclerotic process

Finally it should be re-emphasized that arterial disease should not be considered in terms of lipoids alone to the exclusion of other well recognized factors such as mechanical stress infection vascularity of the vessel wall physico-chemical characteristics of the ground substance (*see* Chapter 28) *etc* Saphir and Gore[71] have recently stressed the possible etiologic role of infection in the coronary disease of young people The work of Meyer[72] and of Faber[73] suggests that the connective tissue changes in atherosclerosis may be independent of the lipoid deposits Blumenthal Lansing *et al* [15, 18] hold that calcification of elastic tissue ( elasto-calcinosis ) is a normal accompaniment of the aging process and is also a prerequisite for the formation of intimal plaques This would not of course explain the well known atheromatosis of infancy [74]

Eventually it is to be expected that some of the new refined methods for studying the fine structure of connective tissue recently reviewed by Gross [75] will be applicable to the problem of arteriosclerosis

**Summary** —It appears clear that much is yet to be learned about the early lesions of atherosclerosis the factors governing permeation of the intima of arteries by lipids and the metabolism of cholesterol and other lipids with particular reference to the physical chemistry of their colloidal state in the blood It seems possible that the level of serum cholesterol or at least the colloidal state of cholesterol in the blood is related in some way to the pathogenesis of atherosclerosis but it is not clear that dietary cholesterol has much influence on the level of blood cholesterol Meanwhile the popularity of cholesterol should not be allowed to lead to neglect of other pathogenetic factors It is possible that alterations in the ground substance may be of much greater importance (*See below and* Chapter 28 )

**Etiology of Atherosclerosis in Diabetes** —As noted above there can be no question that diabetics are prone to develop atherosclerosis at an earlier age and to a greater extent than other persons The reasons for this situation are difficult to assess

It was pointed out in earlier editions of this book that fluctuations in blood sugar concentration might aid in producing swelling of the intimal ground substance through changes in osmotic pressure *In examining biopsies of the skin of diabetic patients in this laboratory it has been found that in those with high blood sugar concentrations the connective tissue of the corium may be slightly edematous and basophilic strongly resembling the change seen in the intimal ground substance in early atherosclerosis* ... very similar

*Unna*[76]

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Much attention has been given to the level of blood lipids particularly cholesterol as a possible etiological factor in the atherosclerosis of diabetes As noted above, it is both affirmed and denied that a relationship exists between the level of the blood cholesterol and the incidence of atherosclerosis Certainly the diabetic is sometimes subject to an elevation of blood lipids and various lesions such as xanthoma have been considered to be related to this elevation However the evidence is conflicting in that frequently the most severe degrees of atherosclerosis may be found in patients with relatively mild diabetes Also it is not clear that well controlled diabetics have any striking aberrations of the serum cholesterol[77 78] Perhaps some of the recently described methods of doing fat tolerance tests[79] and chylomicron counts[80–82] may cast light on this phase of the subject

Wilens[40] has pointed out that overweight is common in all the important conditions that are believed to be associated with the atherosclerotic process (e g diabetes hypertension) He shows that there is a distinct correlation between obesity and the presence of atherosclerosis His study like those of Keys[40–42] suggests that constitutional and other factors must be considered in any analysis of the relation between lipid metabolism and atherosclerosis

Pomeranze and Kunkel[83] found about 50 per cent of 273 diabetics to have a total lipid level above 750 mg per cent which they considered the upper limit of normal Although cholesterol phospholipid and neutral fat were all increased the increment was greatest for neutral fat They found a general correlation between arteriosclerosis and high total lipid high cholesterol or reduced phospholipid/cholesterol ratio They concluded that determination of cholesterol levels alone could not be used as an indicator of total lipid levels Also there was no evident relationship between severity or duration of the diabetes or its control age sex or weight of the patient or size of liver and the total lipid level

Of particular interest is the surprising finding of Duff *et al* [84 85] that alloxan diabetes tends to inhibit experimental cholesterol atherosclerosis in the rabbit These investigators found that the factor most consistently associated with the inhibition seemed to be the presence in the sera of the diabetic animals of a greater amount of neutral fat in proportion to the level of cholesterol than in the normal controls and suggested that this may be due to mobilization of body fat connected with the diabetic state Their findings would seem to emphasize again the necessity of considering the concentrations and interrelations of *all* the lipids of the blood instead of singling out one such as cholesterol

Scarcely any arteriosclerosis has been described in animals in connection with experimental diabetes Allen and Lisa[86] report a dog diabetic for twelve years following partial pancreatectomy and controlled by diet and insulin No vascular lesions were found at autopsy

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Bell[37] has made an extensive study of a large autopsy series of diabetics with particular reference to vascular lesions He emphasizes the high incidence of coronary disease of gangrene of the extremities and of renal arteriolosclerosis (including intercapillary glomerulosclerosis) in diabetes His studies led him to conclude that the frequency of severe vascular lesions increases with the duration of diabetes in those who die before the age of sixty years He also concluded that the development of severe vascular lesions is independent of the severity of the diabetes The last conclusion would apparently be contested by Root and co-workers[88,89] who in several papers present evidence that vascular lesions are likely to be more severe in young diabetics of long duration and that maintenance of good control of the diabetes tends to cut down the incidence and severity of these complications Wilson Root and Marble[89] surveying a series of 247 patients whose diabetes began between the ages of eighteen months and thirty years and had been in existence from ten to thirty four years found in general a lower incidence of vascular calcification and retinitis in those patients under good or excellent control as opposed to those under fair to poor control However it should be noted that they were able to place only 7 patients (2 9 per cent) in the excellent control group

In summary it would appear that the reasons for the increased incidence of atherosclerosis in diabetes are quite obscure Conceivably the newer *work on lesions of the capillaries may shed some light on disease of the larger arteries* Involvement of vasa vasorum might contribute to formation of atheromatous plaques One may look for contributions from the newer studies on the connective tissue ground substance and the physiology of capillary walls (*See* Chapter 28)

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## Chapter 10

# GANGRENE

GANGRENE is one of the most serious complications of diabetes both from the nature of the lesion itself and from the wide path it opens to septic invasion of the body. Among 859 fatal cases of diabetes 144 cases of gangrene developed. Twenty nine were accompanied by generalized sepsis and there were 6 cases of gas gangrene.

TABLE 29.—ONE-HUNDRED FORTY FOUR CASES OF GANGRENE AMONG 859 DIABETIC AUTOPSIES

| | Number | Coma | Generalized sepsis | Gas gangrene |
|---|---|---|---|---|
| All cases | 144 | 13 | 29 | 5 |
| Cases over 15 years duration | 29 | 0 | 7 | 0 |
| Duration unknown | 4 | 0 | 0 | 1 |

Bell[1] from a study of autopsy protocols at the University of Minnesota concluded that the diabetic process intensifies and accelerates to an astonishing degree vascular changes which are present to a lesser extent as part of the aging process in the extremities of nondiabetics. He states "If only the gangrene due to atherosclerosis is considered then gangrene develops nearly forty times as frequently in diabetic as in nondiabetic subjects."

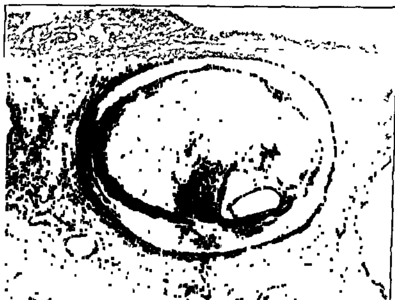
**Embolic Gangrene.**—Gangrene due to embolic occlusion of the larger vessels is the same in diabetic as in nondiabetic persons. No. 438, an arm from a diabetic aged sixty-one years showed embolism and secondary thrombosis of the axillary artery. There was slight medial calcification but no intimal change.

The youngest case of embolic gangrene in diabetes that we have seen occurred in Dr Brigham's case.[2] Sudden gangrene of the left foot developed in a boy aged nineteen years who was in diabetic coma precipitated by pneumonia. In the article in which this case is reported 52 others have been collected from the literature.

**Gas Gangrene.**—Only 6 cases of fatal gas gangrene have been encountered in 859 autopsies. Since the infectious feature of this condition is the most important it is discussed more fully in Chapter 8.

**Diabetic Gangrene.**—It is frequently impossible to tell from the vessels alone whether a given specimen is from a diabetic or nondiabetic.[3] However generally speaking the extent of involvement and especially the atheromatous thickening of the intima may be expected to be greater in

the diabetic Allen, Barker, and Hines[4] prefer the term "arteriosclerosis obliterans" for the type of vascular disease affecting the lower extremities They state, however, that atheroma is the most important component of the lesion



Duration of diabetes 4 3 years



Fig 67 —Lower portion of posterior tibial artery from same case as Figure 66 showing complete occlusion by intimal proliferation and only slight involvement of media, some calcification being present

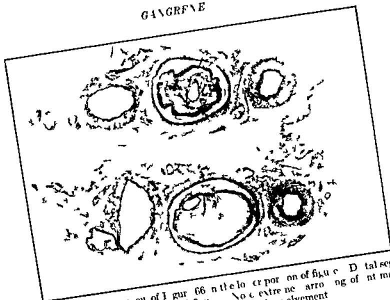


Fig 68 Low power v ew of I gur 66 n the lo er por on of figu e D tal segm of artery shown upper 1o o of figu No e extre ne arro ng of nt ma th ver m nu e amo nt of lum lef Almost no me l al nvolvement

Fig 69 I x reme med al cal fi at on an l narro ng of l men b nt mal prol ferat on

There is one striking difference between arteriosclerotic and diabetic gangrene—the former is more likely to be dry, the latter more commonly moist, although this distinction seems to be less marked since the introduction of antibiotics. The final occlusion of larger arteries is usually sud-

of a collateral circulation and anemic necrosis results. McKittrick and Root[5] illustrate excellently the absence of collateral circulation. As they point out, many diabetics show this type of gangrene.



Fig. 70.—Roentgenogram of injected leg after amputation. Case No. 27117, aged seventy years. Diabetic gangrene. Very extensive collateral circulation present. Note occlusion of dorsalis pedis artery. (McKittrick and Root[5])



Fig. 71. Early diabetic gangrene of foot and osteomyelitis. Case No. 37554. Aged seventy-four years. (McKittrick and Root[6])



Fig. 72.—Gangrene due to embolic occlusion. (McKittrick and Root[6])

Lindbom[6] has published a beautiful arteriographic study of vascular disease in the extremities

Arterial occlusion in the typical diabetic gangrene is a gradual process at least at first—a progressive encroachment on the lumen of the artery by intimal thickening not infrequently showing heavy deposits of lipoid Hence there is time for collateral circulation to develop This is well shown in Figure 70 While there may have been pain and disability during the process of readjustment eventually a delicate point of equilibrium is reached where the combined blood supply from both main vessels and collaterals is just sufficient for the ordinary needs of the limb Any unusual stress will tip the balance There will be insufficient blood supply to maintain life of the tissues under abnormal conditions too much to permit mummification Moist gangrene results and all too often leads to generalized sepsis

On this basis we may say that the typical diabetic gangrene is preventable Injury and particularly infection can be prevented Dirt ill fitting shoes carelessness all are simple all inexcusable in properly taught patients (and only the diabetic with knowledge of how to care for his special hazards and disabilities may defeat the disease) but nevertheless they cause many cases of gangrene

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## Chapter 11

# THE HEART

A NATURAL sequel of the prevalence of arteriosclerosis in diabetic patients is the very frequent occurrence of myocardial injury due to coronary disease. A striking proportion of diabetic patients die of cardiac injury with or without coronary occlusion and infarction. While in the interpretation of the severity of chronic myocardial lesions the personal equation may enter (some individuals regard a given degree of fibrosis as more severe than others would) nevertheless there are four unequivocal lesions concerning the interpretation of which there can be no question. These are (1) a fresh infarct of the heart (2) a healed infarct of the heart with or without aneurysmal dilatation (3) rupture of the heart which is practically always due to infarction (4) coronary occlusion. As may be seen by reference to Table 37 in Chapter 19 these lesions are appallingly frequent in diabetic patients.

The statistics of infarction are widely variable and depend somewhat on the type of practice from which the material is drawn.



Fig. 73.—Heart showing healed and fresh infarcts. Note adherent thrombus in left ventricle. Female aged fifty nine years. Duration of diabetes fifteen years.

Ophuls[1] in 3000 autopsies of which 34 were on diabetic patients found 18 cases of recent infarct and 8 of healed infarct Seven cases showed aneurysms of the left ventricle and in 2 the aneurysmal sac had ruptured

In 1000 consecutive unselected autopsies at the Mayo Clinic 49 infarctions were found [2]

Clawson [3] in a survey of 30 265 autopsies, concluded that about 4 per cent of the deaths could be attributed to coronary sclerosis

The occurrence of infarcts and coronary occlusion in the present series is summarized in Table 30

TABLE 30 —INCIDENCE OF INFARCTS OF HEART AND CORONARY THROMBOSIS IN 761 DIABETIC AUTOPSIES

| | *Fresh* | *Healed* | *Fresh and healed* | *Total* | *Coronary thrombosis without infarction* |
|---|---|---|---|---|---|
| | | *Male* | | | |
| Number of cases | 27 | 35 | 21 | 83 | 5 |
| Average age years | 64.5 | 60.3 | 60.2 | | 59.0 |
| Average duration years | 14.3 | 12.5 | 10.6 | | 10.7 |
| Duration unknown | 1 | 4 | | | |
| | | *Female* | | | |
| Number of cases | 45 | 24 | 26 | 95 | 10 |
| Average age years | 61.2 | 63.3 | 63.9 | | 68.8 |
| Average duration years | 10.8 | 13.5 | 11.0 | | 11.0 |

Thus 178 cardiac infarctions (24 per cent) and 15 additional coronary thromboses without infarction were found in 761 diabetic autopsies

Forty-seven cases had weathered one or more myocardial infarcts before the one that proved promptly fatal On the other hand 15 cases died of coronary thrombosis without time for the development of demonstrable myocardial infarctions

That diabetes is not merely an incidental feature in these cases is illustrated by the duration of the diabetic process long antedating the cardiac condition The average duration is twelve years thus giving ample time for the abnormal metabolic conditions to affect the vessels

Reference to Table 37 in Chapter 19 shows that fully 43 per cent of the deaths in these long duration cases were directly attributable to disease of the coronary arteries Many others of course had severe coronary disease

In our series of the 178 infarcts of the heart 54 per cent occurred in females

Nathanson [4] in reviewing 100 diabetic autopsies found extensive coronary disease in 52 per cent of diabetics above fifty years of age as against 8 per cent in general autopsy material

Root Bland Gordon and White[5] compared 349 diabetic autopsies with 3400 nondiabetic autopsies and found coronary occlusion to be much more

frequent in the diabetics and to occur almost as frequently in diabetic women as in diabetic men.

Stearns, Schlesinger and Rudy[6] found significant coronary disease in approximately three fourths of 50 diabetic hearts studied by Schlesinger's injection technique. Among diabetic women over forty they found coronary artery disease to be as common as among diabetic men. This meant that in their diabetic series coronary occlusion increased twofold in men and eightfold in women.

The most extensive study of the incidence of coronary artery disease in diabetes is that of Clawson and Bell.[7] They reviewed a series of 50,000 autopsies and found that fatal coronary disease was about twice as frequent in diabetic as in nondiabetic males and three times as frequent in diabetic as in nondiabetic females. They also concluded (in common with other writers) that the incidence of disease of the coronary arteries in diabetic women is almost as high as it is in diabetic men, in contrast to the marked preponderance of the disease in nondiabetic men over nondiabetic women. In their series about 4 per cent of the deaths due to coronary disease in males and 14 per cent of those in females were associated with diabetes.

Millard and Root[8] found that 89 per cent of a series of 106 diabetics showed coronary sclerosis of some degree.

Liebow and Hellerstein[9] summarizing several papers from the literature record figures for the post-mortem incidence of "marked" coronary sclerosis in nondiabetics ranging from 8.2 to 37 per cent and for diabetics from 45 to 74 per cent. Obviously differences in the observers' judgments as to the presence of "marked" or "severe" coronary disease must account for a good deal of the variation in these figures. The same authors present another table showing the post mortem incidence of coronary thrombosis varying according to different reports from 2 to 23 per cent for nondiabetics and from 19.6 to 64 per cent for diabetics. Here again observational differences must be important and it is noteworthy that the high *figure of 64 per cent was obtained by use of Schlesinger's injection technique* which is recognized as disclosing a higher percentage of coronary occlusions than any other method (*see* Fig. 78).

One of the youngest deaths from cardiac infarction included in our series of autopsies was a patient of Dr. Joslin, Case No. 1794. When she was twenty-five years of age diabetes developed. Under insulin treatment she had permitted her weight to increase from 67 to 135 pounds. At the age of thirty three years, eight years after the onset of diabetes, she developed intense precordial pain after breaking diet. Death occurred in a few hours after several Cheyne-Stokes attacks. At autopsy she showed fresh infarction of the apex of the left ventricle and the interventricular septum. The left coronary artery was practically completely occluded, barely admitting a horsehair in its first portion. The right coronary artery showed numerous atheromatous plaques. Both of the coronary orifices were surrounded by

atheromata which extended down on the endocardium beneath the aortic ring The aorta showed extensive arteriosclerosis of the atheromatous type

A typical death from coronary insufficiency occurred in Case No 4079 under the care of Dr Joslin a girl whose diabetes had developed four and a half years before her death at the age of twenty two years She had had occasional attacks of pain over the heart while walking and in the week just before her death had frequently complained of it Her death occurred



[illegible]

suddenly with the typical symptoms of angina while riding in an automobile Her blood pressure had been 120 mm systolic and 90 mm diastolic The Wassermann reaction was negative and there was no history of rheumatic fever or chorea Unfortunately an autopsy was not obtained

Joslin s Case No 2703 died at the age of twenty two years with coronary thrombosis[10] In 1920 at the time of the onset of his diabetes eight and a half years before death the Wassermann reaction was negative and systolic blood pressure was 118 mm and diastolic was 75 mm

Diabetes may be mild and yet lead to serious coronary atherosclerosis A man aged twenty seven years whose diabetes had been present 8 5

years had been kept on a low-carbohydrate high fat diet Death was due to coronary sclerosis and occlusion associated with widespread atherosclerosis[11]

Diabetes is by no means a prerequisite for coronary disease in youth as is shown in the remarkable series reported by Yater *et al*[12] of coronary occlusion in young men in military service Here of course the diabetics were screened out before induction

When we consider chronic myocardial fibrosis criteria are difficult to establish However scattered diffuse scarring of the musculature chiefly perivascular and barely visible in gross was considered slight Possibly these fine scars might be regarded as physiological rather than pathological They are encountered very frequently in the hearts of elderly persons non diabetics as well as diabetics A moderate degree of chronic myocardial fibrosis was diagnosed when fine diffuse scarring was visible to the naked eye and when the microscope revealed frequent small foci of fibrosis The presence of generalized fibrosis both gross and microscopic was considered as evidence of a marked degree of myocardial damage

Fairly closely paralleling these changes so far as severity is concerned and of course underlying them are the changes in the coronary arteries Here again three grades of involvement are considered

1 Slight scattered atheromata or fibrous thickening of the intima with but slight narrowing of the lumen

2 Moderate scattered atheromata with or without calcification or fibrous thickening of the intima the lumen reduced not over one-third

3 Marked numerous atheromata usually with some calcification or fibrous thickening of the intima the lumen reduced more than one-third Actual occlusion may or may not be present

TABLE 31 —MYOCARDIAL SCARRING AND CORONARY DISEASE IN 841 DIABETIC AUTOPSIES

| | *Myocardial fibrosis* | *Per cent* | *Coronary sclerosis* | *Per cent* |
|---|---|---|---|---|
| None | 379 | 45 1 | 234 | 27 8 |
| Slight | 160 | 19 0 | 169 | 20 1 |
| Moderate | 98 | 11 6 | 149 | 17 7 |
| Marked | 146 | 17 4 | 261 | 31 1 |
| Total | 783 | 93 1 | 813 | 96 7 |
| Data missing | 58 | 6 9 | 28 | 3 3 |

Clinically angina pectoris is abnormally frequent in diabetic patients The sex ratio is nearly equal Since angina occurs in young diabetics since the duration of diabetes prior to angina is usually long and since the prognosis is poorer for diabetic patients diabetes itself is in all probability a definite factor in the disease[13]

The relatively short duration of life after the onset of angina pectoris in a diabetic is well explained by the severity of the coronary disease and myocardial change found at autopsy.

The occurrence of hypertrophy of the heart is not infrequent, as is shown by Table 32

TABLE 32 —WEIGHTS OF THE HEART IN 817 DIABETIC AUTOPSIES

| | *Number* | *Per cent* |
|---|---|---|
| 0 to 350 gm | 428 | 52 4 |
| 351 to 500 gm | 299 | 36 6 |
| 500+ gm | 90 | 11 0 |

Three hundred and fifty grams was considered as the upper limit of the normal heart Those weighing over 500 grams were considered markedly hypertrophic



FIG 75 —Roentgenogram of heart from case of infarction and coronary thrombosis No 5142 Male, aged sixty-one years Duration of diabetes one and a half years Note the marked calcification of the coronary vessels, also the hypertrophy of the heart The weight was 520 gm

In the coronary arteries as in other arteries of the diabetic patient intimal changes of the atheromatous type predominate. The involvement is not uniform, an artery may be almost completely occluded at one point and relatively normal at another. As elsewhere the atheromatous deposits tend to become calcified and the arteries may become of pipe-stem character or show spots of calcification. Roentgen ray examination of the excised heart demonstrates the calcification well as shown in Figures 75 and 76. In the living body this method is not of value.

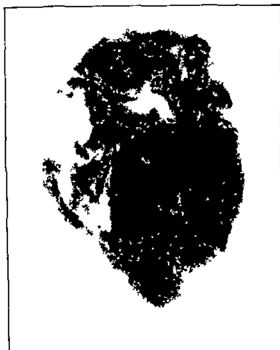


Fig. 76.—Roentgenogram of heart from Case No. 4806. Male aged seventy-six years. Duration of diabetes thirty years. Infarct of heart with rupture. Note calcification of right as well as left coronary arteries.

In addition to the atheromatous type of involvement fibrous thickening of the intima frequently occurs.

While the correlation between coronary arteriosclerosis and that elsewhere in the body is fairly close in the diabetic patient there are wide discrepancies at times, just as are found in nondiabetics.

The most satisfactory method for demonstration of coronary lesions is that of Schlesinger.[14] By the use of colored radio-opaque injection masses, both coronary occlusions and anastomoses can be brought out and the

details subsequently studied by both dissection and histologic examination. Figure 78 shows the roentgenogram obtained from the heart of Case No. 37246, a woman aged sixty four years, duration of diabetes twelve years. There is thrombotic occlusion of right coronary secondary to arteriosclerosis of atheromatous type, several small occlusions in right coronary distribution and the old arteriosclerotic occlusion of the left anterior descending branch. Note also the abundant anastomotic circulation. This heart showed no appreciable myocardial damage and illustrates how well collateral circulation may compensate for gradually developing occlusions.

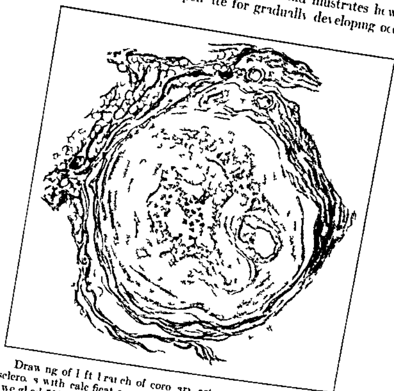


Fig. —. Drawing of left branch of coronary artery from a heart showing marked coronary sclerosis with calcification and occlusion and a healed infarct of the heart. The organ weighed 510 gm. Note extreme involvement of intima. Apparent lumen in the center is due to large mass of fat and cholesterol crystals which has been dissolved out in the process of preparing the section. Male aged fifty-seven years. Duration of diabetes 8 years. Hematoxylin-eosin stain. × 24.

Another serious myocardial injury not infrequently encountered is that of multiple abscesses. In cases as prone to sepsis as those of diabetics it is not remarkable that we should find in this series 7 cases of abscesses of the myocardium.

Diffuse pericarditis was a cause of death in 7 cases and was accompanied by coma in 2 other fatal cases. In 2 additional cases healed pericarditis of the chronic adhesive type was found. Thus 11 of the cases showed evidence of severe past or present pericardial inflammation.



Fig 78 —Roentgenogram of heart injected by method of Schlesinger Note occlusions and anastomoses Female, aged sixty-four years Duration of diabetes twelve years



Fig 79 —Large abscess of the heart from a case of carbuncle with *Staphylococcus aureus* septicemia and numerous abscesses involving heart, liver, and kidneys. Male, aged fifty years Duration of diabetes twenty-three years

In view of the marked susceptibility of diabetic patients to infection and the relative frequency of terminal septicemias, the rarity of bacterial endocarditis and valvular lesions is very striking.

In Chapter 5 the increased amount of glycogen in the diabetic heart was discussed briefly and attention was drawn to the tendency of the glycogen to be deposited in particularly large amounts in the fibers about the margins of infarcted or fibrosed areas, presumably indicating that the muscle fibers at this point were not able to utilize so fully as those elsewhere the amount of glycogen deposited in them. Glycogen apparently exerts no deleterious effect through its presence, although some question may be raised about this point as regards the infants of diabetic mothers (*see* Chapter 23) and glycogen storage disease.

Landing and Bangle[140, 146] have discussed some of the difficulties encountered in making a histochemical distinction between the heart of glycogen storage disease and that of diabetes mellitus, also the evidence suggesting an hereditary linkage between the two diseases. Mowry and Bangle[14] confirm the presence of large amounts of glycogen in diabetic hearts.

Reference has been made in Chapter 7 to the marked fall in serum potassium which may occur in diabetic acidosis. In evaluating the presence or absence of injury from either low or high potassium concentration, the electrocardiogram has proven indispensable. In fact, the electrocardiogram has been shown to be a more accurate index to disturbed potassium metabolism than the level of serum potassium, as indeed might be expected from the fact that potassium is primarily an intracellular ion.[15]

Rodriguez *et al*[18] have described two cases of what they call hypokalemic myocarditis in diabetic coma. The lesions, which consisted essentially of multiple small foci of necrosis together with extensive lymphocytic infiltration, were regarded as due to potassium deficiency on the basis of similar lesions produced experimentally by other workers and a few other human cases.

An admirable review of the cardiac complications of diabetes, including the functional effects of insulin, of high and low potassium levels, and the cardiovascular collapse often encountered in coma, is given by Liebow and Hellerstein.[9]

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## Chapter 12

# THE KIDNEY

The kidney of the diabetic patient may exhibit any of the disease processes found in nondiabetics. However, certain lesions are encountered with far greater frequency in diabetics and two of them (glycogen deposits in the tubules and intercapillary glomerulosclerosis) are almost pathognomonic of the disease.

The renal lesions which frequently complicate diabetes may be listed as follows:

1. Presumably reversible metabolic phenomena
   a. Glycogen deposits in tubules
   b. Fat deposits in tubules
2. Arteriolar sclerosis
3. Intercapillary glomerulosclerosis
4. Acute and chronic pyelonephritis including necrotizing renal papillitis

It should be emphasized at the outset that it is common for two or more of these lesions to be found together in the same kidney in the diabetic. This is particularly true of the last three diseases named and in a case of advanced renal disease it may be very difficult or impossible to assess their relative importance. The younger diabetics who have had their disease for many years almost invariably show a mixture of renal lesions, so much so that one may speak of a "mixed diabetic nephropathy" as characterizing these patients (see Wilson *et al.*[1] Adams[2] and Chapter 19).

*Glycogen deposits* in the renal tubules (so-called "glycogen nephrosis") are discussed elsewhere (see Chapter 5). As noted there, such deposits are presumed to be evidence of terminal glycosuria, an hypothesis which was put forth in earlier editions of this book and which has been given experimental confirmation by Curtis *et al.*[3] The lesion has no known clinical significance other than as an indicator of glycosuria. Experimentally, it can be shown to be reversible by lowering the blood sugar after previous high levels have been maintained.[4]

Fat deposits are also mentioned elsewhere (see Figure 55). Fat appears in the renal tubules under a variety of conditions[5] and is usually regarded as a reversible manifestation of injury to the renal epithelium. As noted in Chapter 7, it has been seen in the diabetic usually in association with acidosis and coma,[6] which in the past was often further complicated by sepsis. Cases without recent coma may also show fat

Recently Mallory[7] has shown that fat vacuolation of the ascending limb of Henle's loop is seen in the majority of cases of traumatic shock surviving one to four days, although he found it only rarely in cases surviving less than eighteen hours. Circulatory collapse with blood pressures of "shock level" is common in diabetic coma,[8] it is probable, however, that the duration of shock in the usual case of coma is insufficient to account for the lesion. Also in the cases of outspoken fat deposition seen in this laboratory the vacuolation was by no means confined to Henle's loop (Figure 55).

Recently a striking case of fatty change of the renal epithelium was seen. The patient, a sixty-seven-year-old woman, had had diabetes for twenty-two years. She died after being in coma for thirty hours, the admission

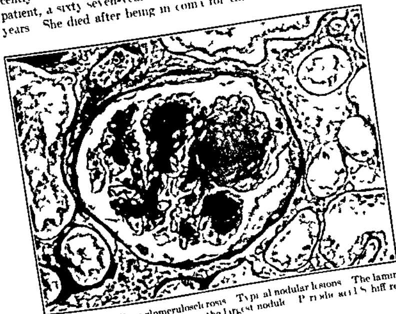


Fig 80.—Intercapillary glomerulosclerosis. Typical nodular lesions. The lamination of the hyaline material is well shown in the largest nodule. Periodic acid Schiff reagent method according to McManus. × 250.

blood sugar having been 720 mg per cent and the $CO_2$ (after some treatment) 22 vols per cent. The patient had a blood pressure in "shock levels" for several hours. The kidneys showed no striking damage to the glomeruli other than a few small foci of fibrosis. The epithelium of the convoluted tubules showed large vacuoles which were shown by frozen section and Sudan staining to be fat.

*Arteriolar sclerosis*, or "benign nephrosclerosis," is common in the diabetic kidney. Anatomically it takes the same form as in the nondiabetic as described by Moritz and Oldt[9] and by others. However, in the diabetic it is likely to appear e[illegible]r and to be more severe, even in cases with apparent good control o[illegible] Some degree of vascular disease in

the kidney has been noted in a large proportion of the diabetic autopsies in this laboratory (*see* Chapter 19), and Robbins and Tucker[10] found arteriolar disease of the kidneys in 55 per cent of their diabetic series. Bell[1] found renal arteriolosclerosis in 72 per cent of diabetics over fifty years of age and states that it is nearly as frequent in nonhypertensive as in hypertensive diabetics. He also found that it increases in frequency with the duration of diabetes in persons under fifty.

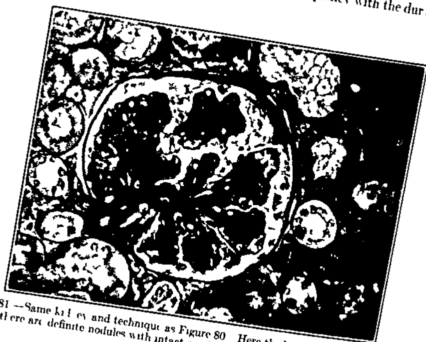


FIG. 81.—Same kidney and technique as Figure 80. Here the lesion is more diffuse but there are definite nodules with intact capillaries on their periphery. × 250.

Death from renal insufficiency due to this lesion, recorded as rare in the earlier editions of this book, is now becoming relatively common as diabetics live longer with their disease. Accordingly arteriolar disease is also seen with increasing frequency in the younger diabetics who have had their disease for a number of years. In such cases the lesion is often part of a widespread involvement of arterioles throughout the body and exhibits itself with distressing results in the retina where it may be combined with capillary and venous lesions (*see* Chapter 13). In the kidney it is often seen in combination with either intercapillary glomerulosclerosis or pyelonephritis or both and may contribute to death from renal insufficiency, a relatively rare situation in the nondiabetic hypertensive patient.

**Intercapillary Glomerulosclerosis.**—This disease has excited increasing interest from both the histopathological and clinical points of view, since its description by Kimmelstiel and Wilson in 1936.[12] One of the original authors has participated in an excellent recent review.[11] As noted there

considerable confusion has arisen as to the histological definition of the lesion resulting in estimates of its incidence in diabetes varying from 20.5 per cent[14] to 63.7 per cent[15].

In its fully developed nodular form the lesion consists of one or more hyaline masses situated in the central part of the glomerular lobule creating the impression of being situated between the capillaries so that characteristically an intact capillary is seen on the margin of the hyaline mass (Figs 80 and 81). The focal character of the lesion and the presence of intact capillary loops in the involved glomerulus are important diagnostic features. Such a histological picture must be clearly distinguished from similar but more diffuse changes which may occur in chronic glomerulonephritis[14,16]. The lesion is usually associated with arteriolar sclerosis of the afferent or efferent arterioles or both. Allen[7,18] considers that sclerosis of the efferent arteriole is significant since he found it only in his diabetic cases. Bell's statement[14] that intercapillary glomerulosclerosis is merely a severe form of arteriolosclerosis with extension into the glomeruli seems to be perhaps an oversimplification of the problem (See Allen[17,18]).

The hyaline nodule may resemble amyloid with routine histological methods but it does not have the staining reactions of amyloid with the exception of an occasional positive reaction with Congo red. With silver stains with McGregor's modification of Mallory's aniline blue stain and with the periodic acid method of McManus[19] it may sometimes be shown to have a laminated appearance (Fig 80). Frozen sections may show variable amounts of lipid. Allen[17,18] also found this type of hyalin to be more resistant to tryptic digestion than nondiabetic hyalin. McManus[19] found that it was removed by pectinase from acetone fixed tissue.

The literature contains considerable dispute regarding the histogenesis of the process. Chief disagreement centers around whether the initial lesion is truly intercapillary. Allen[17], Bell[14] and Laipply *et al*[16] contend that the change arises by a splitting of the capillary basement membrane and Allen would apparently prefer to think of the process as intramural. McManus[20] holds that the hyaline material is deposited in the intercapillary space of Zimmermann. Much of the difference of opinion depends upon whether one accepts the presence of intercapillary connective tissue at the periphery of the normal glomerular lobule. Bell[14] appears to deny its existence but Kimmelstiel and Porter[13] quote Zimmermann as having convincingly demonstrated its presence and at the same time suggest that it may be impossible in any given section to determine how far out into the lobule the intercapillary tissue extends. Fahr[21] drawing an analogy between the structure of the glomerular tuft and the tubule considers the lesion under discussion as extracapillary glomerulosclerosis. Kimmelstiel and Porter[13] point out that the concepts of Allen, Bell and Fahr represent differences in interpretation not of observation and suggest that the term intercapillary glomerulosclerosis be retained since it serves well for descriptive identification.

Bell[14] has made what appears to be a useful distinction in describing two forms of the disease namely, nodular and diffuse The nodular type is the classical picture described above the one most easily recognized and most characteristic of diabetes[18] The diffuse form which is not clearly delineated consists in a diffuse thickening of the capillary basement membrane formed by splitting of said membrane According to Bell[14] it may occur alone or together with the nodular form His series showed an incidence of the nodular lesion in 15.9 per cent of diabetics and the combined nodular and diffuse in 29.2 per cent As noted by Kimmelstiel and Porter[13] the diffuse type would seem to be susceptible of varying interpretations It seems likely that this accounts largely for the extraordinary variation in reported incidence of intercapillary glomerulosclerosis in diabetes For instance the remarkably high figure of 63.7 per cent given by Laipply *et al*[15] is accounted for by their inclusion of "one plus" lesions characterized by focal fibrosis of the majority of glomeruli

In unpublished studies in this laboratory,[27] attempts have been made to confirm the findings of Laipply *et al* with the aid of a few slides kindly sent by Dr Laipply for comparison After study of various types of glomerular change in nondiabetics as well as diabetics it was concluded that it was very difficult to be certain of the specificity of the minimal changes described by Laipply *et al*, as representing the early stage of the process Our own estimate of the incidence of the disease in diabetics approximates the figure of 17 per cent arrived at by Kimmelstiel and Porter[13] from a careful survey of the literature This figure would certainly be higher if the disease were not as noted above so commonly complicated by arteriolar nephrosclerosis or pyelonephritis or both in which cases the diagnosis must often remain in doubt Also when one considers only the younger patients who have had their disease for many years the incidence mounts to a very high figure (*see below*)

It appears likely that the nodular form of the disease is practically specific for diabetes although it is possible that rare cases have been observed in nondiabetics In such cases it is not always clear that diabetes has been positively excluded by an adequately prolonged period of observation glucose tolerance tests and the like McManus[20] notes that glycosuria and even hyperglycemia may disappear with the onset of renal insufficiency in intercapillary glomerulosclerosis (*See also Zubrod et al*[20a])

Kimmelstiel and Porter[13] agree with Henderson *et al*[22] that certain cases of chronic glomerulonephritis may show lesions indistinguishable from intercapillary glomerulosclerosis The latter authors have presented a comparative clinical study emphasizing certain points of difference between the two diseases

Robbins and Rogers[16] have reviewed 100 proven cases of inter[illegible] glomerulosclerosis and 229 cases of dial[illegible] They concluded that all [illegible] sclerosis had diabetes

[illegible] apparently

history revealed ... delineation ... hey feel can ... diabetes m... phrosclerosis of the nodular type of intercapillary ... be differentiated from similar lesions occurring in ... and chronic glomerulonephritis and conclude that in the nodular form the lesion is a specific discrete histologic entity occurring only in diabetes.

Experimental reproduction of the lesion was achieved by Lukens and Dohan[24] in a single dog which had been maintained in a diabetic state for five years by administration of an extract of the anterior lobe of the pituitary gland. Their illustration of the lesion appears convincing. More recently Mann and Goddard[25] have found in rats made diabetic with alloxan a lesion similar to, though by no means identical with, the human disease. The earliest sign is described by them as a thickening of the basement membrane followed by deposition of hyaline acellular material between the capillary loops with, in contrast to the human lesion, eventual adhesions of the tufts to one another or to Bowman's capsule. There is also, again in contrast to the human lesion, proliferative activity on the part of endothelial and epithelial cells in the glomerulus.

The lesions described by Mann and Goddard[25] are probably essentially similar to those described by Foglia *et al*[26] in rats made diabetic by partial pancreatectomy. In neither case can the experimental disease be regarded as a reproduction of the human lesion.

It seems generally agreed that the incidence of the lesion is more closely related to the duration than to the severity of the diabetes, and our own observations conform to this opinion. Some authors[1, 3, 27] find a correlation between duration of diabetes and incidence and severity of the lesion; others[5] do not.

There seems to be a widespread and, we think, erroneous impression that intercapillary glomerulosclerosis is rare in young diabetics. On the contrary, it appears to be common, at least in those of long duration. (*See also* Dawson and Platt[28].) In a small series of young diabetics of long duration observed in this laboratory, intercapillary glomerulosclerosis appears to be present in almost 100 per cent, the few in which it cannot be clearly recognized representing cases in which it may be obscured by other severe lesions as noted above. Indeed, renal disease has become a major problem in these young patients (*see also* Millard and Root,[29] Mann, Gardner and Root,[30] Wilson *et al*,[1] and Adams[2]). According to this finding one might consider the renal lesion as related to the severity of the diabetes, since the younger patients often have the more severe diabetes.[31] However, many years seem to be required before the renal disease is manifest in these cases.

The time required for development of the lesion is unknown. Apparently it may appear fairly rapidly, as indicated by the interesting case of Derow and Schlesinger.[4] Their patient, a sixty-one-year-old woman, had had

diabetes for eight years before her death. Four years before death a nephrectomy had been done. Careful study of sections of this kidney revealed only a single nodular lesion in a single glomerulus, which the authors are inclined to discount because of its lack of association with any axial thickening of the glomerular tuft. Two years before death the patient developed massive albuminuria and at autopsy showed a severe nodular form of intercapillary glomerulosclerosis in the remaining kidney. The authors suggest that the patient may have had the lesion for only two years, coincident with the albuminuria.

There appears to be no correlation between intercapillary glomerulosclerosis and hyalinization of the islets of Langerhans in the pancreas. However, a striking correlation with retinal changes has been noted. Henderson *et al.*[73] found retinopathy in 68.8 per cent of cases of intercapillary glomerulosclerosis as opposed to 22.8 per cent in cases of diabetes without intercapillary glomerulosclerosis. Dolger[33] found that retinopathy tended to appear approximately thirteen years after onset of diabetes, whether in the third or fourth decade, and to be associated in 50 per cent of cases with hypertension and albuminuria, most of the latter being presumed to have intercapillary glomerulosclerosis. Studies of this sort moved Kimmelstiel and Porter[13] to suggest that retinopathy of the diabetic type should be considered part of the clinical symptom complex associated with the renal disease.

The clinical recognition of intercapillary glomerulosclerosis is perhaps more difficult than was thought in the first few years of study of the disease. Robbins and Rogers[16] conclude that not more than 40 per cent of cases can be correctly diagnosed on clinical grounds. Contrary to earlier expectations, it appears that a fully developed nephrotic syndrome can be expected in only about 10 per cent of cases. Hypertension, another feature stressed in the first case reports, is absent in as many as 40 per cent of some series (Henderson *et al.*[73]). The diagnostic value of finding anisotropic lipoids in the urine is stressed by Rifkin *et al.*[81]

Kimmelstiel and Porter[13] sum up the clinical findings as follows: "With the exception of chronic glomerulonephritis, it can be stated that diabetes occurs in almost all cases of intercapillary glomerulosclerosis. Hypertension is found in approximately sixty per cent. Significant albuminuria found in the majority of cases (approximately two thirds), edema of the

that by correlating several of the above findings with the patient's age, a fairly accurate diagnosis may be made in some cases." One can say, however, that in a patient fifty or more years of age with chronic diabetes, nephrotic edema and albumin in the urine with high blood cholesterol, one can safely make the diagnosis of intercapillary glomerulosclerosis with approximately 100 per cent accuracy. They add that

glomerulonephritis may be excluded by repeated Addis counts which may be expected to reveal hematuria not found in intercapillary glomerulosclerosis.

The above statement should perhaps be modified to the extent that intercapillary glomerulosclerosis is probably more closely correlated with duration of diabetes than with the age of the patient. As noted elsewhere, the incidence of intercapillary glomerulosclerosis in young diabetics of long duration is extraordinarily high, both on clinical and pathologic grounds. Rather sudden onset of marked albuminuria, together with rise in serum cholesterol, is often noted in these patients.[30]

The *pathogenesis* of the disease remains obscure. Prolonged diabetes appears to be the *sine qua non*. Hypertension, although commonly found in association with it, cannot be held accountable since undoubted cases occur in the absence of hypertension. Recently Wilens *et al.* have made a study of frozen sections stained with Sudan IV in 21 cases of the disease and find a statistically significant larger amount of fat in the glomeruli in this condition than in any of the other main varieties of renal disease, in diabetes alone, or in diabetic hypertensive patients without intercapillary glomerulosclerosis. Although in a few instances they found large amounts of glomerular lipid in other types of renal disease, the deposition of fat in these conditions bore no constant relation to the severity of the renal lesions. In intercapillary glomerulosclerosis, however, the quantity of glomerular fat was directly proportional to the severity of the lesions in most instances. From these data and from the distribution of the lipids within the hyaline masses and between the glomerular tufts, they conclude that deposition of fat in the glomeruli may be of primary importance in the development of the lesions of the disease. We have confirmed the presence of lipid droplets in the glomeruli but have not made a quantitative study of the phenomenon.

The possible etiologic relation of the lesions of intercapillary glomerulosclerosis to the capillary aneurysms of the retina, especially in relation to the polysaccharide content of the lesions, forms a fascinating subject for speculation (*see* Chapters 13 and 28).

**Pyelonephritis.**—The frequency of urinary infection in diabetes is well recognized.[36] In a series of 196 autopsied cases, Sharkey and Root[37] found purulent infection of some part of the urinary tract in 18 per cent. Robbins and Tucker[10] found acute pyelonephritis to be four and a half times more common as a cause of death among diabetics than in nondiabetics. In their series of 307 diabetic patients it was the sixth commonest cause of death, causing 21 of the fatalities. They found some evidence of acute pyelonephritis in 1 out of every 5 autopsies on diabetics.

This conforms to experience in this laboratory. Even now, when acute pyelonephritis is being controlled more and more with the antibiotics, evidence of healed lesions corresponding to the classical description of Weiss and Parker[38] is found with great frequency.

Recently a particular type of acute pyelonephritis known as *necrotizing renal papillitis* has received increasing attention In the series of 307 cases of diabetes reported by Robbins and Tucker[40] it occurred once in every four cases of acute pyelonephritis and was the severest form of the disease accounting for 16 of the 21 deaths in that group

The lesion has been recognized for years, but has recently been the subject of three detailed reports[39 40 41] which emphasize clinical as well as pathological features



Fig 82 — Necrotizing renal papillitis showing the infarct like patches of necrosis in the pyramids Photograph by courtesy of Dr Stanley Robbins

junction of the pyramid and the cortex As the lesion progresses these abscesses become confluent resulting in a complete necrosis of the terminal two thirds of the pyramid Grossly the lesion resembles an infarct of the cortex but is likely to be yellow brown or yellow green with a peripheral paler zone which in turn is often surrounded by a red congested border The more usual type of acute pyelonephritis may be found in the cortex in varying degree The process is frequently but not always bilateral and in most cases all the pyramids of the affected kidney are involved (Fig 82) Eventually portions of the papillae may break off or sequestration of the whole papilla into the pelvis may occur with associated hematuria and

renal colic (Fig 83) A single case of an apparently healed lesion is described by Edmondson *et al*[40]

The pathogenesis of the lesion remains obscure While common in diabetics it is seen also in nondiabetics with obstructive lesions of the urinary tract No particular bacteria are constantly found although *E coli* and *Staphylococcus aureus* are frequent The infarct like necrosis is thought to be associated with impairment of the already rather feeble capillary blood supply of the papillae Small hyaline thrombi are commonly seen in these vessels



Fig 83 —Necrotizing renal papillitis Low power The necrotic tissue of the papilla has separated like a sequestrum

A somewhat similar lesion was produced experimentally by Mallory[42] by ligation of one ureter in rabbits and intravenous injection of bacteria No significant lesions appeared in the nonobstructed kidney but on the obstructed side an acute pyelonephritis with necrosis of papillae was observed Muirhead *et al*[43] produced something similar by aseptic ureteral ligation in dogs However they offer no evidence to explain the high incidence in diabetes without obstruction Robbins and Angrist[41] call attention to the earlier work of Borland and Jackson[44] who described necrosis of the renal pyramids with calcification in rats on a fat free diet The relation of this work if any to the human lesion is not clear The same applies to the experimental medullary necrosis induced with vinylamine by Mandel and Popper[45]

Clinically, the disease carries a high mortality and should be recognized as early as possible and treated vigorously. Edmondson *et al*[40] suggest that the disease should be suspected in the following types of diabetic patients: (1) those with sepsis in whom urinary symptoms or findings suddenly develop, (2) those who go into coma rapidly with nitrogen retention but without an antecedent history of pyelonephritis, (3) those with low-grade pyelonephritis who suddenly become worse, (4) those with hematuria or renal colic, (5) those who fail to rally from severe acidosis with ade-

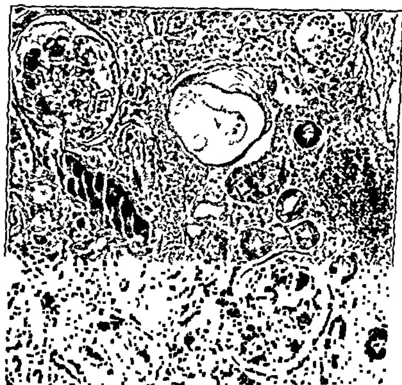


FIG 84.—Mixed diabetic nephropathy in a young diabetic of long duration. There is evidence of intercapillary glomerulosclerosis, arteriolar sclerosis and pyelonephritis. × 150

quate treatment and whose nonprotein nitrogen continues to rise even after the blood sugar and carbon dioxide have returned to normal, and (6) those with characteristic roentgenographic findings.

Robbins *et al*[22] emphasize that the disease may appear as an acute fulminating febrile illness without premonitory signs or else as an acute severe exacerbation of a chronic urinary infection.

The pyelograms may be diagnostic. The following changes have been emphasized[40]: (1) a deformity of the tip of a calyx similar to the usual

pyelonephritis (2) a picture of excavation extending into the cortex, (3) ring shadows may be seen in the necrotic papillæ, (4) extreme dilatation of the concave tip of the calyx, (5) tips of sequestrated papillæ may appear as free masses in the renal pelvis

**Summary** —The kidney from a diabetic of long standing especially one in the younger age group may be expected to show a mixture of lesions usually arteriolar sclerosis intercapillary glomerulosclerosis and pyelonephritis (Fig 84) Although intercapillary glomerulosclerosis is practically confined to diabetes clinical recognition is difficult and furthermore it is the mixture of lesions that is characteristic of the diabetic kidney Therefore the common clinical tendency to refer to any diabetic with signs of renal disease as having Kimmelstiel Wilson disease would seem to be unfortunate

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# Chapter 13

# THE EYE

THE question as to whether the diabetic eye shows any characteristic or pathognomonic changes is still disputed. However, certain lesions do occur more frequently in diabetics than in the general population. Some of these lesions are better studied with the slit lamp or ophthalmoscope than by histological techniques and have been particularly well summarized by Waite and Beetham[1] to whose paper the reader is also referred for a discussion of the functional alterations.

**Eyelids.**—Waite and Beetham[1] reported a slightly increased incidence of xanthelasma* and of squamous blepharitis in their series as compared with normal controls.

**Conjunctiva.**—Waite and Beetham[1] found a slightly increased incidence of conjunctivitis in the diabetic series. Cultures in 40 cases showed a predominance of *Staphylococcus aureus* in most instances.

**Cornea.**—In the series referred to[1] wrinkles of Descemet's membrane were found to be definitely more frequent in the diabetics. They also appeared at an earlier age than in the controls. The incidence of arcus was the same in both groups.

**Iris.**— It is an old observation that in the operation for iridectomy in diabetic patients, iris pigment is readily set free and makes the aqueous dark and cloudy, or alternatively, in an operation for cataract a part of the pigment layer may come away with the lens.[2]

The cause of this phenomenon, known for many years to ophthalmic [illegible] n of the pigment [illegible] ul and illustrated [illegible] glycogen in the vacuolated cells. He stated that the normal eye is free from glycogen but that in inflammation, circulatory disturbances and diabetes, deposits of glycogen may be found in the iris, the corneal epithelium, the retina, and the optic nerves. The presence of vacuoles and glycogen in the pigment layer of the iris in diabetes has been confirmed by others and Villani[3] has produced similar changes in rabbits given intraperitoneal injections of glucose over a period of twelve to thirty-four days. Although the presence of glycogen may not account entirely for the swelling of the pigment epi

* Xanthelasma [illegible]

thelium and the depigmentation of the iris frequently seen in diabetics it seems likely that it is a major factor

**Lens** The generally accepted manifestation of ocular pathology in diabetes mellitus is the prevalence of cataract Noted first by *Rollo*[1] one hundred and forty years ago it is mentioned by practically all textbooks of ophthalmology Only in recent years has there been some doubt cast as to whether cataract in the elderly person varies in the diabetic and in the nondiabetic There is definite evidence that cataract in the juvenile is far more frequent in diabetic patients than in nondiabetic patients

Waite and Beetham[2] in their careful study of ocular pathology in diabetes examined 2 459 patients diabetic and nondiabetic They found in



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those over twenty years old that a total of 60 per cent of the diabetic patients (3 047 eyes) showed lens opacities of all types whereas among 526 eyes of nondiabetics 57 per cent showed opacities Not only was the total number of lens opacities the same in diabetics and nondiabetics but there was virtually no difference in the proportion of complicated cataract found 6 per cent among 4 001 diabetic eyes and 8 per cent among 914 nondiabetic eyes

*On the basis of Danish statistics* Anthonisen[3] found that depending upon age from 15 to 202 times the number of cataracts in diabetics were operated upon in the Rigshospital as would be expected from the prevalence of cataract in the general population

In a study of the chemical constitution of the cataractous lens of adult type in both nondiabetics and diabetics the findings as given by Carey and Hunt[10] are approximately the same. Thus in 30 nondiabetic and 13 diabetic cataractous lenses the cholesterol content was 525 and 511 mg. per cent respectively, the calcium 30 and 35 mg. per cent respectively. However, there was a distinct difference in the phosphorus content, there being in the nondiabetic 19.7 mg. per cent of nonlipoid phosphorus as against only 6.2 mg. per cent in the diabetic. The cholesterol content of the cataractous lens is approximately 20 per cent greater than that of the normal lens and the calcium content, roughly three times that of normal lens or normal blood.

Cataract has been a striking and frequent complication of experimental alloxan diabetes in rabbits and rats. (*See* Chapter 25.)

Waters[11] studied the biochemical changes in the lens of the eye during experimental diabetes in rabbits and found that although the glutathione content of the lens remained within normal limits the total sulfhydryl content decreased within four hours after alloxan and remained low as long as two years. He concluded that the decrease must be due to a decrease in the sulfhydryl groups of protein. Not all these rabbits got cataract but the experiment showed that early chemical changes occur in the lens within a short time after alloxan.

One feature of cataract in the diabetic patient is seen in the juvenile patient where there is development of a distinctive type of opacity, distinctive in the sense that it does not occur in normal individuals although it is indistinguishable from the cataracts induced by various of the metabolic disorders and dinitrophenol. These opacities occur in the anterior and posterior cortical portions of the lens and resemble a chemical precipitate of flocculent nature. They have been described as diabetic cataracts by Schnyder[12] and as snowflake opacities by O'Brien, Molsberry and Allen.[13]

An extensive discussion of cataract may be found in the monograph by Bellows.[1]

**Retina.** For many years the question as to whether a specific diabetic retinopathy exists has been a matter for debate among ophthalmologists.[2] Recently the opinion seems to be growing that in many diabetics including some without hypertension and without evidence of arterial or arteriolar disease there occurs an ophthalmoscopic picture characterized by small punctate and irregular hemorrhages often associated with dilatation and tortuosity of the retinal veins with sharply defined hard white exudates and sometimes ending in the late stages in a type of retinitis proliferans in which many newly formed vessels are found. Several recent writers have laid particular stress on the small punctate hemorrhages (many of which are probably microaneurysms) as representing the earliest detectable lesion in diabetic retinopathy. As a result of this increased attention has been devoted to the study of the capillaries and venules rather than the arterioles as the probable site of the early damage in diabetes. This point was

emphasized by Waite and Beetham[1] who found deep retinal hemorrhages in 18 per cent of 1915 visible fundi in diabetics. Flynn[15] postulated a dilatation of the capillaries with consequent slowing of blood flow (which he called "prestasis") leading to diapedesis of erythrocytes and the formation of small hemorrhages. He felt that this condition was more clearly related to hyperglycemia than to any other factor.

Particular interest attaches to the work of Ballantyne[16,17] who described microaneurysms of the retinal capillaries as the earliest lesion in diabetic retinopathy.* These aneurysms are described as about 50 to 60 micra in diameter, occur chiefly in the perimacular region and are perfectly round. In fact many of the so-called punctate hemorrhages probably actually represent this lesion. Ballantyne describes them also in flat unstained

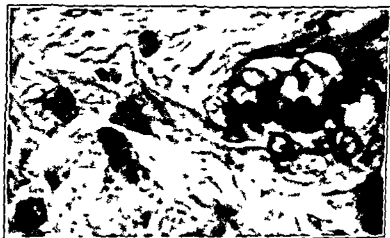


*Fig. 86.—Diabetic retinopathy. The capillary passing from the left and slightly downward suddenly balloons out into a microaneurysm containing red blood cells.* × 1000

specimens of the formalin fixed retina and in histological section. He states that they may have thickened hyaline walls and may show endothelial proliferation. Thrombosis and deposition of fat in the endothelium may also occur. We have found it difficult to locate these lesions by gross examination of autopsy specimens by Ballantyne's method, presumably because the retinal vessels in the usual cadaver are fairly well emptied of blood. However, in occasional fortunate sections we have encountered lesions which seem to conform to his description (Fig. 86).

The study of these capillary aneurysms has been greatly altered by Friedenwald's[8,9] useful discovery of the value of the periodic acid Schiff reagent method of Hotchkiss and McManus when applied to whole mounts

* As noted by Sherrill,[10] retinal microaneurysms were described and illustrated by Edward Nettleship in a case of "glycosuric retinitis" reported by Mackenzie in 1879.[11]

of the retina By this method, which has also proved so valuable in studying the renal glomerulus (*see* Figs 80 81), the capillary basement membranes are stained an intense purple red When the whole retina is stained dehydrated cleared and mounted flat without sectioning a beautiful delineation of the capillary network is obtained and it becomes clear that the lesions described by Ballantyne are indeed aneurysms which often occur in clusters and which may have thickened, hyaline walls (Fig 87 and Plate III) These tiny aneurysms which doubtless in large part represent the punctate hemorrhages of the clinical literature seem to be most common



Fig 87 — Diabetic retinopathy Capillary microaneurysms demonstrated by the periodic acid-Schiff reagent method applied to whole mount of retina (Dr Jonas Friedenwald courtesy of Am J Ophth 32 487 1949)

in diabetes being found only occasionally in other conditions[18,19] although Ashton,[20a] using injection methods finds small peripheral aneurysms in about a third of nondiabetic eyes The interesting question as to whether these lesions occur to any great extent in other tissues of the body has not been answered A few have been described in the conjunctiva[19,21] and nail beds[19]

Friedenwald[19] and also Ashton[22] cite the well known association of this type of lesion with intercapillary glomerulosclerosis (*see* Chapter 12) and speculate as to whether there may be any pathogenetic relationship Friedenwald[19] makes the interesting suggestion that both may represent a disturbance in the metabolism of mucopolysaccharides This concept is enlarged upon in Chapter 28

Ballantyne[17] also emphasizes the possible importance of venous stasis in the production of capillary dilatation and hemorrhage. Tortuosity and beading of the veins are fairly common and the occurrence of phlebosclerosis has been described particularly by Gibson and Smith.[23] They stress especially a hyaline thickening of the media of the retinal veins. Similar observations are reported by Agatston[24] and O'Brien and Allen.[25] The latter authors describe venous varices in the retina as being more common in diabetics than in patients suffering from arteriosclerosis and renal dis-

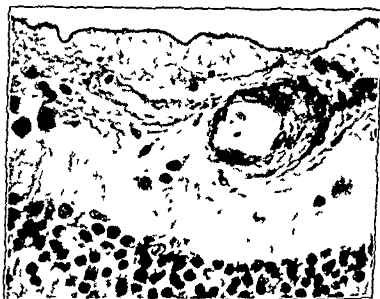


Fig. 88.—Eccentrically thickened and dilated retinal vein. × 500



Fig. 89.—Extreme proliferative reaction in wall of small retinal vessel, probably a vein. × 500

eyse (Figs 88 89) It should be emphasized here that arteriolar sclerosis is often seen particularly in the choroidal vessels but is similar to that found in nondiabetics

The possible existence of an increased capillary fragility in diabetes as an explanation of the frequency of retinal hemorrhage has been suggested by Hanum[26] Wagener[27] and others and has led to the treatment (without clear-cut results) of patients with vitamin C and various flavone derivatives such as vitamin P (citrin) hesperidin and especially rutin Rodriguez and Root[5] found increased capillary fragility in 40 per cent of 100 unselected diabetic patients practically all of those having retinitis showed an in-

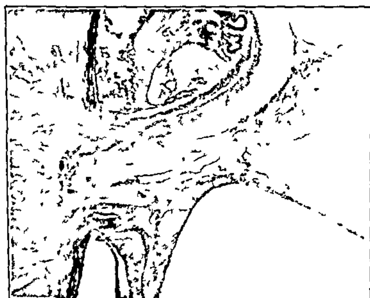


Fig 90 Retinitis proliferans in a diabetic eye The optic nerve is at the left [illegible] nerve head [illegible] vitreous [illegible] (above)

creased fragility The fragility could be brought to normal by giving rutin

therapy with rutin

The hard white exudates seen ophthalmoscopically in diabetic retinopathy are sometimes referred to simply as hyalin Friedenwald and Koyanagi[28] regard them as masses of inspissated protein presumably resulting from transudation of plasma through capillary walls The [illegible]

regards them as characteristic of diabetes. *Deposits of fat and cholesterol* sometimes in phagocytes may also be seen.

Retinitis proliferans, which consists essentially in the growth of a *connective tissue membrane from the retina into the vitreous*, may be the unfortunate end result of diabetic retinopathy,[36] although it is not specific for diabetes. The pathogenesis is obscure, although it seems to be associated with the organization of repeated hemorrhages. Some authors emphasize the frequent association of this condition with the renal complications so often seen in diabetes of long duration. Klien[37] *distinguishes two types of*



*Fig. 91.—Retinitis proliferans.* Same as Figure 90 but [illegible] to show detail of the proliferating vascular connective tissue membrane. Dark granules represent [illegible]. × 125

retinitis proliferans. The first is due to inflammatory or traumatic alterations in the retinal vessels and is characterized by being located anywhere in the fundus and by the formation of a dense, opaque connective tissue membrane rich in cells but containing few blood vessels. The second, *which is the type found in diabetes*, originates at or near the optic disk and is characterized by a profusion of newly formed blood vessels appearing in advance of any noticeable amount of connective tissue, and frequently showing a brush-like arrangement with coils or loops in the vitreous return-

ing to the disk (Figs. 90-91). Klien[37] considered the latter type as due to degenerative vascular disease leading to extensive anastomosis.

From the standpoint of the general pathologist, retinitis proliferans would seem to be most easily explained as equivalent to an organizing hematoma in other tissues. Its histologic pattern would then presumably be due to the characteristics of the vitreous and its frequency in diabetics would be the result of the multiple retinal hemorrhages so common in these patients.

Another change not infrequently seen in the late stages of diabetic retinopathy is occlusion of the central retinal vein either by thrombosis or intimal



Fig. 92.—Fibrin nets in the retina in a diabetic. This may be seen in albuminuric retinitis in nondiabetics. × 250

proliferation. This leads often to the formation of a peculiar connective tissue membrane on the anterior surface of the iris (Fig. 86).

In the late stages, and particularly in cases with advanced vascular disease of the kidneys, the lesions in the retina may be complicated by those of so-called "albuminuric retinitis" with marked arteriolar sclerosis in both the retina and choroid and the deposition of "fibrin nets" (Fig. 92). These lesions are beautifully illustrated in color by Schieck.[38]

**Nerves.**—The only change which appears to be distinctive for diabetes is the deposition of glycogen in the corneal and optic nerves mentioned by Best[4] and fully described and illustrated by Hoffmann.[39]

**Summary** In summary the following ocular changes may be said to be characteristic of though not necessarily specific for diabetes (1) vacuolation of and deposition of glycogen in the pigment epithelium of the iris (2) capillary hemorrhages and especially microaneurysms in the retina (3) dilatation of retinal veins with phlebosclerosis (4) the deposition of dense protein rich transudates in the retina (5) retinitis proliferans (6) occlusion of the central retinal vein with formation of a vascular connective tissue membrane on the anterior surface of the iris

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# Chapter 14

## THE NERVOUS SYSTEM

ALTHOUGH diabetes mellitus is intimately connected with the nervous system (emotional glycosuria, decreased carbohydrate tolerance during periods of mental anxiety), demonstrable changes in the nervous system are almost entirely due to complications of the disease rather than to diabetes itself.

The most striking changes are those secondary to vascular disease. Cerebral hemorrhage is much less frequent, relative to other types of arteriosclerotic disorders, in diabetics than in nondiabetics. The same holds true for cerebral infarction. Among 818 deaths of this series, only 29 were due to cerebral arteriosclerotic changes. However, DeJong has reiterated the point of view that many of the neurologic manifestations of diabetes may be attributable to vascular disease.

Sections of the brain in Case No. 4006, dying as a result of massive intraventricular hemorrhage, are shown in Figures 93 and 94. This case was a woman, aged sixty-seven years, whose diabetes had been present 21 years. The hemorrhage was secondary to rupture of the left middle cerebral artery. Of course, changes of this type are in no sense peculiar to diabetes.

From the brains which we have personally examined, and from the reports in the literature, there are no changes distinctive of diabetes other than the abnormal glycogen deposits in certain cases of diabetic coma. We have not seen the droplets of glycogen described by Geipel[2] as occurring in the pyramidal cells of some cases of diabetic coma. Casamajor[3] has described the presence of droplets of glycogen in the perivascular lymph spaces of the brain in coma cases, although these are frequently present in the infectious diseases as well. When the glycogen is present in large amounts, droplets may be present in the glia cells. One of us (S. W.) has seen this type of glycogen deposition in 3 diabetic brains, 2 from coma cases. Other aspects of cerebral change will be discussed in Chapter 22.

The findings of Medwedeff and of Dillon, Riggs, and Dyer in the brain in cases of coma are mentioned in Chapter 7. The specificity of such changes may be questioned.

A series of brains from the present series is now being studied by Dr. S. P. Hicks. His incomplete results suggest that there are no constant changes. Minor changes are, of course, hard to evaluate, since diabetics are subject to such a variety of complicating diseases, especially of the blood vessels. The findings of Vonderahe[4] and of Morgan *et al.*[5] of a reduced number of ganglion cells in the paraventricular regions have not been

confirmed, although Chiari[6] has claimed to find degenerative changes in this part of the brain in a single case.

**Diabetic Neuropathy.**—While neuritis particularly of the lower extremities is common in diabetes the underlying lesions are poorly understood. Paresthesia and anesthesia are not rare. Apparent trophic disturbance



FIG. 93.—Massive intraventricular hemorrhage from middle cerebral artery. No. 4606. Female, aged sixty-seven years. Duration of diabetes 2.1 years.

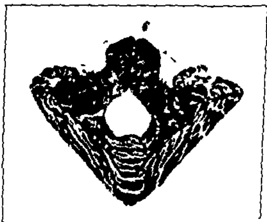


FIG. 94.—Hemorrhage in fourth ventricle from same case as above.

may occur.[7] Woltman and Wilder[8] have collected 35 cases from the literature and have reported 10 additional cases. In 3 of these the spinal cords were examined and showed only slight lesions most easily explained on an arteriosclerotic basis and not similar to the degenerative changes of pernicious anemia. The posterior columns were the most involved. They

found in the peripheral nerves degeneration allegedly associated with marked sclerosis of the intraneural vessels and maintained that atherosclerosis is the most important factor in the lesions of nerves in diabetics. However their illustrations do not show the lesions usually regarded as atheromatous but rather what appears to be a mild concentric thickening of the walls of small vessels.

Other investigators[9,10,10a] have re-emphasized the rôle of sclerosis of the small vessels in diabetic neuritis. The evidence is not convincing however and it is not apparent that an adequate control series has been studied. Furthermore neuropathy of this type is not common in the advanced generalized arteriolar sclerosis of hypertensives, as one might expect if it were purely on a vascular basis.

Fraser[11] found a man aged thirty-six years whose diabetes had been present 1.25 years with neuritic symptoms especially in the lower leg to have demyelinization of the posterior tibial nerve.

As Woltman and Wilder[9] pointed out the pre-insulin diabetic neuritis was largely on a cachectic basis. While some[12] have suggested vitamin deficiency as a factor it is hard to think of a diet better provided with antineuritic vitamins than that of the typical diabetic. The role of vitamins is hard to assess.[13,14] Rundles[15,16] points out that vitamin therapy without control of the diabetes seems to have made some cases worse.

A group of nerves removed in this laboratory has been studied chemically.[17] The diminution of lipoids roughly paralleled the increase in arteriosclerosis. About one-half the cases had neuritic manifestations though

TABLE 33 AVERAGE VALUES WITH RESPECT TO ARTERIOSCLEROSIS DIABETIC COMPARED WITH NORMAL NERVES[18]

| *Arteriosclerosis* | *No. of cases* | *Phospholipid per cent* | *Cholesterol per cent* | *Cerebroside per cent* |
|---|---|---|---|---|
| 1+ (slight) | 1 | 3.85 | 1.15 | 1.60 |
| 2+ | 6 | 1.09 | 0.64 | 0.69 |
| 3+ | 12 | 1.67 | 0.60 | 0.77 |
| 4+ (advanced) | 1 | 1.40 | 0.45 | 1.46 |
| Normal average | | 4.40 | 1.30 | 1.73 |

frequent abnormalities of circulation might have played an important part in the symptoms. Efforts to correlate diabetic neuropathy with the chemical constituents of the blood[19,20] have shown some elevation of the phospholipids in those patients showing neuropathy.

*However the pathologic aspect of the problem has yet to be settled* since clinical observation has shown diabetic neuritis to be independent of arteriosclerosis.[21,22] Often occurring in young inadequately treated diabetics it may last for months only to disappear completely with adequate therapy. Thus far we have not had opportunity for histologic study of such a case. The pathology appears to be practically unknown.[16]

Sections of spinal cord have been studied in a number of our cases without definite abnormalities but we have not had material from a case of tabes diabetica

The prompt recovery in Major's[3] case suggests that the cord changes cannot always be severe

Griggs and Olsen[24] described three types of cord involvement as occurring in diabetes (1) degeneration of the motor cells of the brain stem and spinal cord (2) degeneration of the intramedullary portion of the dorsal root fibers with secondary system degeneration producing a lesion like tabes dorsalis and (3) funicular necrosis of the posterior columns occurring especially in the cervical and upper dorsal segments of the cord They report an example of the last type The significance and pathogenesis of these lesions are obscure and their specificity may be questioned

The rapidity of recovery in some cases of diabetic neuritis practically precludes any marked pathological change This is evidenced by the case of Root[5], a middle aged woman who had double wrist drop and double toe drop but made a good recovery in six weeks time when she was given effective therapy for her diabetes

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## Chapter 15

# THE PITUITARY

In the effort to find some explanation for the occurrence of diabetes in the absence of pancreatic pathology, clinical observation has led to the suggestion that pituitary disease is responsible for a certain number of cases. Marie[1] in describing acromegaly as a clinical entity noted that diabetes occurred in 2 of his first 4 cases and with the accumulation of case reports a definite relationship between these diseases became evident. The association of pituitary tumors and glycosuria had already been noted by Loeb.[2]

Cushing[3] in an admirable series of clinical observations and experiments produced strong evidence of the association of glycosuria with excess secretion of the pituitary and conversely the association of an abnormally high carbohydrate tolerance with hyposecretion.

*Presumptive evidence of the relationship of diabetes and the pituitary* gland are the studies by White[4] and Joslin[5] emphasizing the occurrence of diabetes among children who are taller than the ordinary and who show other features suggesting pituitary abnormalities. Rosenbusch[6] however was not able to confirm this.

Table 34 gives some idea of the great frequency of diabetes or better glycosuria in acromegaly.* The subject has been reviewed by Coggeshall and Root[7] who also compared the organ weights of acromegalics and diabetics and found in general a predominance in favor of the former.

TABLE 34. FREQUENCY OF GLYCOSURIA IN ACROMEGALY

| *Author* | *Cases of acromegaly* | *Number showing glycosuria* | *Per cent* |
|---|---|---|---|
| Hansemann[8] | 97 | 12 | 12 |
| Hinsdale[9] | 130 | 14 | 11 |
| Williams[10] | 21 | 6 | 29 |
| Borchardt[11] | 176 | 71 | 40 |
| Anders | 88 | 16 | 18 |
| Rosenberger[12] | 196 | 82 | 42 |
| Cushing and Davidoff | 100 | 25 | 25 |
| Coggeshall and Root[7] | 153 | 5[illegible] | 31 |

* It must be emphasized that there is considerable overlapping of cases in this table. For instance Coggeshall and Root include in their figures the 100 cases of Cushing and Davidoff which they reviewed again finding the incidence of glycosuria higher than the original authors had thought.

One important difference between ordinary diabetes mellitus and that associated with hyperpituitarism is the tendency of the latter type to remain mild and even to regress. Two cases of Cushing (cited by Colwell) are known to have regressed after relief of pressure from the pituitary tumor.

The possibility that changes in the pituitary may hinder insulin formation by the pancreas is slight. Colwell[15] has collected 15 cases of acromegaly showing pancreatic changes which may be classified as follows: fibrosis, atrophy, hypertrophy, pancreatitis (hemorrhagic and suppurative), hyalinized islands, diminution of number of islands, and hyperplasia of island cells. Certainly no distinctive effect on the pancreas can be detected here. Root[16] has found no significant differences in the pancreatic weights of acromegalics with and without diabetes. It must be emphasized again that differential cell counts (*see* Chapter 3) have not been done in these cases.

Anterior lobe extract has been used by Young[17] to produce a permanent experimental diabetes in dogs (*see* Chapter 25). His results have been confirmed by others including Best.[18] Sections of a well preserved pancreas of one of Best's dogs examined by one of us (S. W.) showed marked hydropic change of the island cells. This is of special interest as assay of this pancreas showed it to contain no insulin.

Hydropic change (probably glycogen deposition—*see* Chapter 3) is characteristic of the early reversible stage of pituitary diabetes called idiohypophyseal by Young. If injections are continued the pancreas may be permanently damaged and a permanent ("metahypophyseal") diabetes ensues. This is discussed more fully in Chapter 25.

Doctor Young very kindly sent material from the pituitaries and pancreases of his dogs 28 and 41. One of us (S. W.) failed to find marked histological changes in either the pancreases or the pituitaries although the insular epithelium showed apparent hydropic change. An incomplete cell count from the pituitary of dog 41 showed acidophils 43.2 per cent, basophils 5.9 per cent, and chromophobes 50.9 per cent. Some of the chromophobes were larger than usual. He reported in one of his dogs complete absence of normal island cells.[19]

Firmly established as is the influence of the anterior pituitary on carbohydrate metabolism, the role of this gland in human diabetes has yet to be clarified.

Attempts have been made to explain the ordinary type of diabetes on the basis of pituitary lesions. Fry[20] has described adenomatous proliferation of eosinophilic cells in the anterior lobe, areas of cellular degeneration, and colloid invasion as characteristic of diabetes mellitus. Kraus[3] found similar lesions in the pituitary glands of 10 diabetics between fourteen and forty-one years of age and in 2 of 12 cases over forty-one years of age. The pancreas was negative in only 1 case, however. He has found the pituitary in diabetes often small, even two thirds normal size.

Kraus[22] has described hydropic change of the basophilic cells in 2 diabetics whose pancreases also showed hydropic change. In the majority of the cases studied by him[23] there was a net reduction in the acidophilic elements when the size of the pituitary and the size and number of the acidophilic cells were considered, although he also mentions adenoma-like masses of acidophils.

These findings have been accepted by many writers.[24] However, it is interesting to note that when Kraus presented his findings at the meeting of the Deutsche Pathologische Gesellschaft in 1923,[25] disagreement was voiced in the discussion that followed by at least two pathologists. The findings of Labbe and Petresco[6] tended to confirm those of Kraus.

Cunz[7] distinguished two kinds of chromophobe cells in the anterior pituitary, the stem cells and chief cells (Hauptzellen). The former type is the progenitor of all cells, chromophobe as well as chromophil, and the latter type he apparently regarded as a differentiated but nongranulated cell. In 11 of 15 diabetics, most of them elderly, Cunz claimed to find an increase in these chief cells, usually associated with a diminution in acidophils. He likens the situation to a "shift to the left" or maturation arrest such as may occur in the bone marrow. He also recorded an increase in interstitial tissue in several cases.

If it be true that the growth hormone is the important one in experimental pituitary diabetes (*see* Chapters 25 and 26) and if it does play a role in some cases of human diabetes, one might expect an increased number of acidophils rather than the decrease noted by Kraus in most of his cases. In this connection Kraus[28] simply states, "Though the findings are not entirely uniform, there cannot be any doubt that, while hyperfunction of the pancreatic islets causes progressive changes in the hypophysis (such as hyperplasia and adenomas), a deficient function resulting in diabetes leads, particularly in experimental animals and in juvenile persons, to retrogressive lesions as described by Kraus." He also suggests that the discrepancy between his data and the negative findings recorded in earlier editions of this book may be due in part to the scarcity of juvenile patients in our series and in part to the effects of insulin treatment.

In a study of a series of 107 pituitary glands Parsons[29] included 7 cases of diabetes and one of hemochromatosis. No significant pathological change was found.

In 20 cases of diabetes Crooke[30] failed to find any hyaline change in the basophilic cells.

Only one instance of moderate increase in number of basophils was found by Hawking[31] among 6 cases of diabetes on which he did differential cell counts by the Rasmussen method.

We have been unable to confirm the findings of Kraus and of others who have reported characteristic findings in the pituitary in diabetes mellitus. As noted below, we have had hardly any acute, untreated cases in young

patients and it is possible of course, that Kraus was fortunate in having a group of such cases before insulin came into general use

Dr Louise Eisenhardt[12] has examined in serial section the pituitary glands of 18 of our cases of diabetes mellitus and random sections from 26 additional cases She found no significant changes Examination of additional random sections in our series has given no result at variance from hers In her group of serially sectioned pituitaries the material was chosen to represent young diabetics whose disease was of long duration (2 cases) adults ranging from forty three to sixty nine years of age with diabetes of short duration and adults ranging from forty-eight to seventy four years of age with diabetes of long duration Some of the patients had no insulin others had received slight amounts of insulin and others were under adequate control One case died in coma 12 showed a mild diabetes and the remainder severe The durations in these cases ranged from one month to eighteen and one-half years Thus this group the glands of which were studied in serial section represents a very fair cross section of the diabetic population except for the omission of acute cases in the young Autopsies have been very few on such cases in

clinical colleagues In the one case

48 91 studied through the courtesy

who died acutely in coma the diabetes having been unrecognized prior to admission to the hospital we were unable to detect any definite changes in size of the gland or in distribution of cell types There was no evidence of necrosis scarring or hyalinization

In the 18 cases mentioned above the pancreases showed wide ranges of appearance Some were completely negative others showed various changes such as diminution in number of islands fibrosis or hyalinization of the islands variation in size of islands and hemochromatosis Fatty infiltration of the pancreas was noted in 5 cases

These pituitary glands showed no constant significant change The most constant findings were posterior lobe invasion by basophilic cells and slight to conspicuous vacuolization of these basophilic cells in the anterior lobe The latter may be comparable to the change noted by Kraus in 2 of his cases associated with hydropic change of the islands of Langerhans Since this vacuolization of the basophils occurred also in the control cases it is not regarded as a significant lesion Basophils with two nuclei were common An adenoma like mass of basophils lay in the anterior lobe in Case 21757 The acidophils seemed to show no significant change in number except in one case that of a young male diabetic where the number was decreased and the cells were small in size Some of the cells contained two nuclei In 8 other cases multinucleated cells were present Small vacuoles were frequently noted in the cytoplasm in 2 cases the acidophils had homogeneous cytoplasm and one cell was found in mitosis

Only one case therefore showed the diminution in acidophils that Kraus has stressed and none showed the adenomatous cell groups

that he mentioned The chromophobes were noted as large in 4 instances but otherwise were not significantly altered except for the existence of vacuoles in occasional cases which gave some of them the appearance of foam cells Nuclear pyknosis was common being present sometimes in all three cell types at times restricted to one or another In 4 cases small foci of necrosis were encountered in the anterior lobe In 3 cases there was a slight increase in interstitial tissue Colloid in the anterior lobe was within normal limits The colloid was particularly conspicuous in Case 20284 mild diabetes of four months duration occurring in a woman aged sixty years The one case of hemochromatosis showed heavy pigment deposition throughout the anterior and posterior lobes The pars intermedia showed colloid in all cases moderate in 9 and marked in 6 Cysts lined by ciliated epithelium were present in 3 cases Similarly no constant changes were found in the posterior lobe Posterior lobe invasion by basophils was noted in 17 of the 18 cases and was marked in 6 and moderate in 5 cases

*We feel the conclusion is justified from these data that the pituitary gland* presents no significant abnormality in diabetes mellitus with the possible exception of rare acute cases in the young

There are two reports of apparent Houssay phenomenon in man *i e* marked amelioration of a pre-existing diabetes seemingly due to destruction of the anterior pituitary by a coincident disease [22,23]

For a discussion of experimental pituitary diabetes *see* Chapter 25 For consideration of the possible etiological role of the pituitary in human diabetes *see* Chapter 26

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# Chapter 16

# THE ADRENAL GLANDS

SINCE the demonstration that removal of the adrenals from a depancreatized animal would lead to amelioration of the diabetes[1,2] and that administration of adrenal cortical extract to such an adrenalectomized depancreatized animal would again increase the severity of the diabetes[4] much additional evidence has been added especially by the work of Ingle[5,6] on rats and that of Forsham *et al.*[7] and particularly of Conn[8,9] in producing a diabetic state experimentally in the human subject by administration of ACTH. Also of note is the work of Kobernick and More[10] in producing hydropic change (glycogen[11]) in the islets with cortisone. Further details of the experimental background for involvement of the adrenal glands in diabetes together with appropriate references may be found in Chapter 25.

Clinical evidence for the participation of the adrenal cortex in human diabetes has been provided in the first place by cases of Cushing's syndrome which is usually associated with either tumor or hyperplasia of the adrenal cortex and in which there is usually impairment of glucose tolerance and insensitivity to insulin as indicated by the glucose-insulin tolerance test.[12,13]

Tumors of the adrenal cortex are commonly associated with diabetes usually of mild degree.[14,15] Shepardson and Shapiro[16] studied 18 cases of adrenal tumor with diabetes and hirsutism ("diabetes of bearded women"); they noted no striking pancreatic changes in 8 autopsies other than one carcinoma and one case of hemorrhagic necrosis; however, specific granule stains were not used. In some cases cure of the diabetes has followed removal of adrenal cortical tumors.[16,17] In such cases it is to be presumed that the diabetic state is accounted for by an overproduction of those cortical hormones which are chiefly concerned with carbohydrate metabolism, i.e., the 11 and 11-17 oxygenated steroids.[18]

Lukens *et al.*,[9] in summarizing 55 cases of tumor or hyperplasia of the adrenal cortex, found impaired carbohydrate tolerance in 49 per cent and well marked glycosuria in 35 per cent. Russi and Blumenthal[20] reviewed 9000 autopsies with reference to the state of the adrenal cortex. Of the total 3 per cent had diabetes. There were 131 cortical adenomas and of these 21 or 16 per cent were diabetic. Of the 21, 16 also had hypertension.

Selye[21,22] has extended his concept of stress to include episodes of hyperglycemia and ketosis following psychic or physical trauma and, in fact, includes diabetes among the "diseases of adaptation." The implication is that oversecretion of adrenal cortical steroids in time of stress is the essen-

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show osteoporosis and an elevated excretion of 11-oxysteroids, thus indicating a compensatory overactivity of the pancreas in order to prevent the hyperglycemia that would be expected. Finally, carrying this reasoning over to diabetic patients, they found, in common with Miller and Mason [32] a somewhat decreased excretion of 17-ketosteroids and an essentially normal eosinophil response to ACTH. Thus they suggest that the activity of the pancreatic islet cells tends to vary directly with that of the adrenal cortex in man.

Also of interest in this connection is the work of Field and Marble [33] who studied the response to stress in 25 diabetics and 13 nondiabetics as measured by the eosinophil count on the second postoperative day following major surgery. Ten of the 25 diabetics showed an eosinophil count which was depressed less than 50 per cent of the preoperative level, whereas all 13 controls had more than a 50 per cent fall in eosinophils. Also, 5 of the diabetic patients showed less than a 50 per cent drop in eosinophils in four hours following a dose of 25 mg. of ACTH. (The same patients showed an adequate response to cortisone.) The conclusion was that some diabetics may have a diminished adrenal cortical reserve.

We have not observed any constant gross or microscopic changes in the adrenals in the present series. In a series of 50 cases the adrenal cortices have been measured in autopsy slides by the method described by one of us[34] and no significant change found. Vartiainen[35] found no change in the weight of the adrenals in his cases.

**The Medulla.**—The adrenal medulla has never excited great interest as a possible causative factor in diabetes, although the classical work of Cannon[36] established the hyperglycemia of sudden stress as due to an increased secretion of epinephrine. The reasons for this lack of interest have been apparently, as Ricketts[15] states, the transitory nature of the response to epinephrine, the failure to produce diabetes by long-continued injection of epinephrine, and the lack of effect of ablation of the adrenal medulla on the course of experimental diabetes. However, as Ricketts[15] also points out, human disease has excelled the laboratory in providing evidence that epinephrine may be diabetogenic. Since 1941 seven cases of pheochromocytoma have been reported in which the associated diabetes was cured or markedly ameliorated by removal of the neoplasm [37–43]. One of these cases had as much as 85 units of insulin daily. The actual mechanism of production of diabetes by these tumors is obscure and may perhaps be attributed to the action of epinephrine on the "pituitary-adrenal axis" [44] with secondary overproduction of cortical steroids. Thus the diabetes would be essentially of the adreno-cortical type.

The state of the pancreas in such cases of reversible diabetes due to adrenal tumors is, so far as we are aware, totally unknown except for the single case cited by Lukens [43] in which the β cells showed marked degranulation (early hydropic degeneration?) but had normal-appearing nuclei. It

tral factor (*see also* Hinkle *et al* [3] and Chapter 26) The enlargement and other changes in the adrenal cortex recently described in the course of alloxan diabetes[24, 25] are perhaps to be regarded as nonspecific effects of stress due either to toxic effects of the alloxan or to the diabetic state itself In fact in any discussion of the role of the adrenals in human diabetes the possibility must be kept in mind that any changes encountered in the adrenals (such as those of Russi and Blumenthal [26] mentioned above) may possibly represent the nonspecific *response* of the organism to the stress of the disease state rather than having any primary etiological significance

The rare concurrence of diabetes and Addison s disease [26, 27] is of interest Usually the former has preceded the latter, and in some cases amelioration of the diabetes has been observed with the onset of adrenal cortical insufficiency thus presumably reproducing the animal experiments mentioned above Balfour and Sprague[17] describe two such cases In one when the Addison s disease was treated only with desoxycorticosterone acetate the concentration of blood sugar decreased during fasting the glycosuria and ketonuria were of only moderate intensity However when the Addison s disease was treated with 11-dehydrocorticosterone (compound A) or 17 hydroxydehydrocorticosterone (compound E) the diabetes was greatly intensified and severe ketosis developed Another interesting human experiment was performed by Green *et al* [28] who removed in two stages not less than 98 per cent of all adrenal tissue in a twenty nine year-old diabetic with severe hypertension The patient had been diabetic since the age of six Before operation the daily insulin requirement was 70 to 85 units and the blood pressure was 260/140 Six weeks after operation when the patient was being maintained on 15 cc of cortical extract daily the insulin requirement was 25 units and the blood pressure was normal

Hinerman[29] has made the interesting observation that the islands of Langerhans are uniformly hyperplastic in cases of Addison s disease and that while all cell types appear increased the $\alpha$ cells show the greatest increase Hinerman explains this hyperplasia as due to the removal of an inhibitory influence normally exerted in the presence of adrenal cortical hormones We have been able to confirm his findings in a few cases but feel that the explanation is by no means clear and may be a nonspecific effect such as the $\alpha$ cell hyperplasia which Ferner[30] obtained by repeated injections of insulin in the guinea pig

Recently evidence has been brought forward that adrenal cortical function may actually be diminished in diabetes Wilson *et al* [31] have demonstrated that patients with Addison s disease respond with marked diabetic symptoms to a dose of cortisone which would have minimal effects in a normal person They suggest that the Addisonian patient is habituated to a relative islet cell insufficiency as a compensatory protection against hypoglycemia They also point out that by no means all patients with adrenocortical hyperfunction have manifest diabetes although they may

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is obvious that pancreatic biopsies adequately fixed and stained in cases of this type would be of extraordinary interest

In the present series we have encountered 3 cases of pheochromocytoma Unfortunately all were discovered unexpectedly at autopsy so it is not known whether the diabetes could have been reversed by extirpating the tumors All were males around sixty years of age and all had had diabetes for at least six years In no case was the presence of the tumor suspected clinically Two died in postoperative shock probably because of the presence of the tumor the operation having been done for an unrelated condition One was an incidental finding at autopsy No significant changes were found in the pancreas of any of the three

Sayers *et al* [49] have made the interesting observation that removal of the adrenal medullas from a strain of rats with hereditary diabetes resulted in a normal tolerance to injected glucose while administration of epinephrine to the demedullated animals would again give a diabetic type of glucose tolerance curve

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sugar free She died of nephritis three years later and at autopsy the thyroid showed a marked strumitis with almost complete destruction of the alveoli The head of the pancreas was markedly fibrosed in the body of the pancreas many of the islands were hypertrophied some reaching three or four times the normal size

Another instructive case is cited by Wilder[6] This was a boy who developed diabetes when fifteen months of age For six months he was kept on a fairly rigid diet and then this was somewhat relaxed without any evidence of glycosuria A diagnosis of hypothyroidism was made when he was three years of age and with the institution of thyroid feeding intense glycosuria and acidosis developed The thyroid administration was then discontinued and the tolerance increased When seen by Wilder he was seven years of age and showed typical juvenile myxedema Through administration of thyroid the metabolic rate was gradually raised to − 10 and at that point sugar appeared in the urine As much as 20 grams per day were excreted Although the child gained in weight and strength sugar was present in the urine on a diet with a glucose equivalent of 76 grams and 10 units of insulin daily Balfour and Sprague[7] give several case histories illustrating the effect of hyper- and hypothyroidism on diabetes including one young man who underwent a total thyroidectomy in order [illegible] betes more tractable (This result was indeed accom[illegible] myxedema

TABLE 35 DIABETES ASSOCIATED WITH HYPERTHYROIDISM

| No | Age yrs | Sex | Duration of diabetes yrs | Pancreas: Size | Pancreas: Islands | Pancreas: Acini | Cause of death |
|---|---|---|---|---|---|---|---|
| 4289 | 30 | ♀ | 0 2+ | 30 gm | Negative | Negative | Coma |
| A27 82 | 38 | ♂ | [illegible] | [illegible] | Few fibrosed | Marked fibrosis | [illegible] |
| DA27 12 | [illegible] | ♀ | 6 5 | 70 gm | Negative | Negative | Rheumatic carditis |
| DA26 25 | 43 | ♀ | 2 [illegible] | Normal | Negative | Slight fibrosis | Coma |
| 14291 | [illegible] | ♂ | 1 0 | [illegible] gm | Negative | Moderate fibrosis | Tracheal obstruction |
| 5665 | [illegible] | ♀ | [illegible] | Large | Negative | Carcinoma | Carcinoma of pancreas |
| 73006 | [illegible] | ♂ | 11 | 100 gm | Negative [illegible] | Marked fibrosis | Uremia (pyelonephritis) |
| [illegible] | 42 | ♀ | 3 3 | 60 gm | Slight fibrosis | Moderate fibrosis | Cancer |

[illegible] one case of myxedema and diabetes This man [illegible]

# Chapter 17

## OTHER ENDOCRINE GLANDS

### THYROID

SINCE the presence of hyperglycemia and glycosuria in cases of hyperthyroidism is not rare there is naturally a tendency to connect the disease with diabetes John[1] pointed out that there is no definite relationship between the severity of hyperthyroidism or the height of the basal metabolism and the degree of hyperglycemia It is natural to assume therefore that this is not due to the action of the thyroid itself but is due to a deficiency in insulin formation which is brought to light by the strain of hyperthyroidism

This conclusion seems to be supported by the experimental production of diabetes by thyroid extract in animals already partially pancreatectomized (*see* Chapter 2)

Holst[2] found in 10 autopsied cases of diabetes associated with hyperthyroidism that there was no change in the pancreas in 4 in 6 cases however the pancreas was small and in 4 of these the number of the islands of Langerhans was diminished It is important in this connection to keep in mind the great difficulties facing any estimate of the number of islands present in the human pancreas and the wide range in number of islands known to be present normally[3] (*see* Chapter 3)

Various degenerative changes in the islands particularly necrosis or round-cell infiltration have been reported It is possible to see how hydropic change of the insular epithelium might be present through long continued functional strain and possibly fibrosis may eventually develop in the islands

However other changes are very difficult to associate We have had an opportunity to study the pancreas of several cases of severe and long continued hyperthyroidism when the tissue was obtained very fresh In the pancreas of these cases there was no change demonstrable Garrod[4] however mentioned the occurrence of atrophic lesions of the pancreas in certain cases of exophthalmic goiter

Some evidence has been produced for the occurrence of hypertrophy of the islands in cases of hypothyroidism or after thyroidectomy One of the most interesting clinical evidences of this relationship is the one cited by Rohdenburg[5] in which a woman aged thirty years was found to be glycosuric and in spite of treatment could not be made sugar free When she was forty years of age the menopause began and after this she became

In the female varying degrees of atrophy of the ovaries and uterus may occur largely proportional to the duration and severity of the diabetes. Most of the reports are in the older literature of the pre-insulin era. In two-thirds of Kraus' cases the ovaries were small, half or less the size of *those of normal women. The development of follicles may be interfered* with.[12] This may be a direct effect of the disease on the ovary or it may be exerted through the pituitary. The former is more likely. The alterations are not specific for diabetes.

Kraus[13] ascribed the rarity of pregnancy in diabetic women first to the regressive ovarian changes leading to amenorrhea and sterility and second to the hindrance to conception offered by the catarrh of vulva and vagina so often present. In contrast with the past rarity of pregnancies in diabetes a large series of successful pregnancies has been reported by White.[14]

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tion Post-mortem examination showed chronic thyroiditis coronary sclerosis and an 80-gram pancreas with some large islands and some showing moderate hyalinization

In the present series we have been able to study 8 autopsied cases of diabetes associated with hyperthyroidism As can be seen from Table 35 the ages range from twenty-nine to sixty-seven years

There is absolutely no characteristic picture in the pancreas In fact the islands were negative in all except 2 cases where their number was apparently somewhat decreased and they showed varying degrees of fibrosis The cause of death in one of the cases was carcinoma of the pancreas but many of the islands persisted uninjured in the midst of the carcinomatous tissue

Total ablation of the normal thyroid has not been worth while as a means of controlling diabetes although sugar tolerance does increase [7,8]

Hyperthyroidism even of long standing produces no lesion in the pancreas and conversely no characteristic lesion of the thyroid is found in diabetes (We have not been convinced that the changes mentioned by Labbe and Petresco[9] are significant ) Changes in the Golgi apparatus and mitochondria of the thyroid possibly indicating hypofunction have been described in alloxan diabetes in the rat [10]

## PARATHYROID

No consistent abnormality of the parathyroid glands has been found in these diabetic patients

## PINEAL GLAND

No abnormality of the pineal gland has been encountered

## THYMUS

In this series no thymic abnormality has been noted Major has encountered 2 cases of apparent thymic enlargement One of these was a girl aged eleven years dying in coma [11] The thymus weighed 50 grams the lymph nodes were enlarged and the pancreatic islands were few

## GONADS

The sterility formerly noted in male diabetics is in all probability secondary to the malnutrition and frequent toxemias and attacks of acidosis of the poorly treated or untreated diabetic We have noted no change beyond cessation of spermatogenesis and some increase in thickness of the basement membrane of the tubules The interstitial cells are unaffected In the adequately treated diabetic patient there is no apparent alteration from the usual picture

islands present in different portions by the rough comparison of those countered in usual routine microscopic sections On this basis 12 cases [illegible]

lative insufficiency of insular tissue becomes actual as the slight factor of fety is reduced by injury from infection and other causes

TABLE 36 —FATAL CASES OF DIABETES UNDER FIFTEEN YEARS OF AGE AT ONSET

*Part A —Deaths Prior to January 1 1930*

| ase No | Sex | Age at death yrs | Duration | Cause of death | Islands of Langerhans | Arterio-sclerosis |
|---|---|---|---|---|---|---|
| 78 | ♀ | 1 5 | 3 4 mos | Coma | | – |
| 76 27 | ♂ | 2 | 1 mo | Coma | Lymphocytic infiltration hydropic change | – |
| s 865 | ♂ | 2 2 | 17 days | Coma | | – |
| 22-3 | ♂ | 2 9 | ? | Coma bronchopneumonia | Hydropic change pyknotic nuclei | – |
| on 2849 | ♂ | 3 8 | 1 1 yrs | Coma otitis media | Pyknotic nuclei | |
| 27 100 | ♂ | 4 | 2 wks | Coma | Lymphocytic infiltration H+ | |
| on 27? | ♀ | 5 8 | 1 9 yrs | Coma bronchopneumonia | Few | |
| 11 23 | ♀ | 6 | 6 wks | Coma | Few | – |
| ol n | ♂ | 6 | 1 3 yrs | Accident fractured skull | Hypertrophy (cobra type) | |
| tarr ow | ♀ | ( | 6 wks | Coma | Lymphocytic infiltrat hydropic change | – |
| 545 | ♀ | 1 | ? | Septicemia | | – |
| ta sfiel l | ♀ | 12 | 5 days | Coma | Lymphocytic infiltration | |
| 949 | ♀ | 12 | 4 yrs | Coma sore throat | | + |
| 21 103 | ♂ | 12 | 1 8 yrs | Coma acute pericarditis | | + |
| 115 68 | ♂ | 13 | 11 mos ? | Coma | Few hydropic change | |
| A22 73 | ♂ | 14 | 1 wk | Coma | Slight fibrosis | + |
| 1904 | ♂ | 15 | 1 6 yrs | Coma | Lymphocytic infiltration | |
| A22 141 | ♀ | 15 | 1 mo | Coma | Few | – |
| 125 | ♀ | 15 | 8 mos | Coma | | |
| U23 10 | ♂ | 15 3 | 8 | Coma | Few | – |
| 41978 | ♂ | 16 | 5 5 yrs | Coma | Slight fibrosis | + |
| 115 102 | ♀ | 38 | 29 | Coma | Slight fibrosis ? hydropic change | + |

*Part B Deaths from January 1 1930 to September 1 1935*

| Case No | Sex | Age at death yrs | Duration yrs | Cause of death | Islands of Langerhans | Arterio-sclerosis |
|---|---|---|---|---|---|---|
| 12490 | ♂ | 8 | 4 3 | Insulin reaction | | M |
| 28346 | ♂ | 12 | 12 | Sepsis | | + |
| 10392 | ♂ | 14 | 8 ? | Pulmonary tuberculosis | Few S+ | + |
| 34637 | ♂ | 14 | ? | Bronchopneumonia coma | | |
| 32878 | ♂ | 16 | 4 9 | Insulin reaction | Few | + |
| 10564 | ♀ | 17 | 2 | Acute glomerular nephritis | H+ | + |
| 21607 | ♀ | 23 | 11 | Meningitis, coma | | + |

M = information missing

## Chapter 18

# THE PATHOLOGY OF DIABETES IN CHILDREN

In children alone may we hope to find pure diabetic lesions uncomplicated by any of the degenerative processes of age. Any changes which we find in the pancreas in children, it is fairly safe to say, are definitely associated with the diabetic process. For this reason it is discouraging, at least from the standpoint of pathology, that many of the pancreases in diabetic children fail to show any definite pathological change.

Of the 22 fatal cases up to 1930 reported in Table 36 but 4 had been treated with insulin for any length of time. A few of the others received insulin when already in terminal coma, they were first seen by a physician. Of the 4 cases treated for any length of time with insulin, one was killed by an automobile, 2 were treated in the dawn of insulin therapy, and one, a negro girl, was treated for six months before death from coma precipitated by a severe sore throat, the duration of the diabetes being four years so far as known.

The cause of death in the diabetes of childhood in this first series was coma. In 5 cases coma was accompanied by infection sufficiently severe to be recognized at post mortem.

In the second series, largely drawn from insulin treated cases, infection is the important factor.

In the third series, while infection is still important, the longer period of survival causes cardiac and renal disease, especially the latter, to emerge as the major factors (*see also* Chapters 12 and 19).

It is noteworthy that among the 25 fatal cases since January 1, 1930, only two have died in coma. The increasing age at death of these childhood cases is also of interest in relation to the effectiveness of modern treatment.

While the pancreas is noted as small in many cases, this observation is uncertain. The size of the pancreas is far from constant even in normal persons, and the temptation is to regard as significant an organ slightly smaller than normal, paying no attention to those in the series that may slightly exceed normal.

Terbruggen[1], however, was impressed by the small size of the pancreas in his juvenile diabetic group.

Quantitative studies of the amount of insular material present in the human pancreas are not yet feasible as routine procedures. The difficulties in the way of enumerating the islands of the pancreas are tremendous (*see* Chapter 3) and in none of this series has it been attempted. However, from a number of sections it is possible to gain some idea of the various numbers

Perhaps the one most characteristic lesion of the islands in diabetic children is lymphocytic infiltration as already emphasized by one of us (S. W.). This lymphocytic infiltration present in 6 cases rarely occurs in older individuals. It strongly suggests the action of some toxic substance or possibly a mild reaction to the necrosis of island cells produced through functional overstrain. The short duration of these cases is very suggestive, in 5 being six weeks or less and in the one case of longer duration being only nineteen months.

Reports of 5 autopsied cases of infants dying of diabetes up to one year of age were summarized by Lawrence and McCance. Four of these showed lymphocytic infiltration of the islands. Two cases were apparently secondary to a generalized cirrhotic or inflammatory process in the pancreas. Lymphocytic infiltration is obviously not a primary lesion of the islands but rather a response to pre-existing injury. Von Meyenburg calls it "insulitis" and suggests that it may be due to functional overstrain ("Ueberlastung").

Hydropic change was positively diagnosed in 7 cases and suspected in two cases of the present series. Hyalinization was encountered four times and fibrosis of the islands eight times. The islands seemed entirely normal from the qualitative standpoint in 13 cases, and normal both in number and histology in 25. However, the newer special stains (see Chapter 3 and Appendix A) were not used.

A fairly typical case is M004, a boy aged fifteen years, whose diabetes had begun 1.6 years before death in coma. This was a pre-insulin case. The pancreas was small. The islands showed marked lymphocytic infiltration but their number was normal. There was no involvement of the acinar tissue. The heart was negative. There was marked glycogenic infiltration of the epithelium of Henle's loops and also of the liver-cell nuclei.

Case 8820, a boy aged fifteen years, died in coma following a sore throat 1.5 years after the onset of diabetes, which had been treated osteopathically. This case is not included in the table as death occurred after the statistical work was completed. Lipemia was marked. The pancreas showed apparent decrease in islands with some hydropic change. The spleen and reticulo-endothelial system showed lipoid histiocytosis. Atheromatosis of the aorta was present. The liver weighed 1,750 grams, showed much glycogen in the liver-cell nuclei and traces in the cytoplasm. Fat was present in moderate amount.

In this series the occurrence of large livers was not striking. Joslin finds hepatomegaly in 5 per cent of diabetic children, and Marble et al. have discussed in detail hepatomegaly as seen in 60 diabetic children.

Atrophy of the pancreas accompanied by pituitary changes has been reported by Kraus in a diabetic boy, aged fourteen years, and a girl aged seventeen years.

Acute pancreatitis should always be suspected in a child dying of coma. The fact that hyaline degeneration, fibrosis, and other irreversible tissue

*Part C—Deaths from September 1 1937 to September 7 1948*

| Case No | Sex | Age at death yrs | Duration yrs | Cause of death | Islands of Langerh… |
|---|---|---|---|---|---|
| 43175 | ♂ | 11 mo | diag. 14 days | Undetermined | Lymphocytic infiltra… Pyknotic nuclei S… H++ few |
| 53319 | ♂ | 7 | 2 5 | Undetermined | Hydropic change |
| 108601 | ♀ | 14 | 10 | Acidosis and coma | |
| 30820 | ♂ | 15 | 1 5 | Coma | * hydropic change …minished in numbe… |
| 63523 | ♂ | 16 | 5 5 | Meningitis | Few |
| 88704 | ♀ | 22 | 15 | Renal insufficiency | S+++ |
| 43829 | ♀ | 23 | 17 | Pulmonary tuberculosis | H+ Few small |
| 80547 | ♂ | 25 | 15 | Operative shock (pyelonephritis) | Few small |
| 46105 | ♀ | 26 | 13 | Renal insufficiency | Few small |
| 80140 | ♂ | 27 | 14 5 | Renal insufficiency resulting from pyelonephritis | Hydropic change Few small |
| 77407 | ♀ | 29 | 16 4 | Undetermined | |
| 74831 | ♀ | 30 | 21 | Undetermined | |
| 80901 | ♂ | 30 | 18 | Cardiac infarct | Abundant |
| 104277 | ♀ | 30 | 23 | Renal insufficiency | Few small |
| 66891 | ♂ | 32 | 24 | Myocardial infarction | Hydropic change S- diminished in numbe… |
| Twins 87068 | ♂ | 32 | 20 | Pulmonary edema | S+ diminished in number |
| Twins 95181 | ♂ | 33 | 23 | Cerebral embolism (cardiac) | |
| 96127 | ♀ | 33 | 18 | Renal insufficiency | |

Moore's[2] case is of great interest A thirteen year-old girl, wh… betes was of six years duration with but little effort at control,

In a case of a young man made available by Dr I D W… only were definite islands and even stars suggesting islands absent, b… of the arterioles showed an extraordinary degree of hyaline thic… Special stains were unsatisfactory, but suggested the presence of some… cells Diabetes had been present for sixteen years and coma had oc… five times He died at the age of twenty-nine years from acute glom… nephritis and bronchopneumonia The liver was very large 3010 … with a total fatty acid content of 3 23 per cent, within normal limit… sulin assay of the pancreas by Dr G T Evans showed none prese… though the same method had demonstrated insulin in the pancreas of… cases of diabetes

Radiographic study of the bones of diabetic children has revealed multiple transverse lines of arrested growth in the long bones in some, probably associated with poor control of the disease.[11]

While diabetes in children is usually severe, it becomes less severe with increasing duration of the disease, and there was one case in this series of which the duration was twenty nine years without the aid of insulin.

Allan and Wilder[12] in a study of 17 cases of diabetes in children which resulted fatally, emphasize the potential severity of juvenile diabetes. Two deaths were due to intercurrent disease and one was moribund when treatment was started—the others were due to inadequate treatment. Unfortunately no autopsies were obtained.

White's[13] careful studies of diabetic children have done much to advance our knowledge in this field.

Priesel and Wagner[14] suggested juvenile diabetes to be a form of congenital insulin insufficiency associated with such degenerative stigmata as absent uvula, dwarfism, and bony malformations.

Lerner[15] has also put forth the concept of a congenital insular deficiency or rather inferiority ("Minderwertigkeit") of the cells of the islands which prevents them from ripening in the normal manner.

The problem presented by infants of diabetic mothers will be discussed in Chapter 23.

White accepts two definite cases of congenital diabetes.[13]

That congenital anomalies[16] may be important in rare instances is suggested by the case of Ghon and Roman.[17] An idiot boy, aged fourteen years, developed diabetes and died. The only pancreatic tissue was a plaque of pancreas within the duodenal wall and bulging into the lumen.

Rosenbusch[18] gives a broad discussion of childhood diabetes from all points of view including the psychiatric. He discusses a possible relationship between juvenile diabetes and von Gierke's disease (glycogen storage disease) suggesting that both may represent disturbances of the autonomic nervous system, i.e., the hypophyseodiencephalic mechanism, the one tending to glycogenolysis, the other to glycogenesis. He seems to think that if one conceives of diabetes mellitus as a dysfunction rather than a hyperfunction of this mechanism, one may correlate with Kraus' findings of small pituitaries and diminished acidophils in cases of diabetes (see Chapter 15). Rosenbusch did not find in his series the acromegaloid attributes observed by White in some of her diabetic children.

In recent years, as juvenile diabetics have survived ten to twenty five years with the disease, it has become painfully apparent that a very high incidence of vascular disease must be expected in this group. Thus Dolger[19,20] found that within a period of twenty five years' duration of diabetes not one of 200 regularly examined patients escaped retinal hemorrhage regardless of age of onset, severity of diabetes, or type of treatment used. He also found that retinopathy usually presaged progressive vascular

degeneration and that 50 per cent of the patients exhibited hypertension and albuminuria at the time of the earliest retinal hemorrhage.

Mann, Gardner, and Root described a disturbingly high incidence of intercapillary glomerulosclerosis associated with a nephrotic syndrome in young diabetics of long duration. In a series of sixteen fatal cases of renal disease complicating diabetes, the average age at onset of diabetes was twelve; the average duration of diabetes at first signs of renal disease was 12 years; the average duration of diabetes at death was nineteen years; and the average age at death was *thirty-two years*.

White and Waskow reported evidence of vascular disease in 92 per cent of 200 diabetics surviving twenty years of the disease. Chute, Adams, and Rosenbusch[15] have commented on the appallingly high incidence of vascular damage in juvenile diabetics between the fifteenth and twentieth years of the disease.

*As may be seen by comparing Table 36 above with Table 37 in the next* chapter, and as emphasized elsewhere, there is a striking preponderance of renal and retinal lesions, i.e., vascular disease affecting chiefly the *capillaries* in those patients whose diabetes begins in childhood. In fact, efforts directed toward preventing these degenerative complications constitute one of the major therapeutic problems in the field of diabetes today.

Considerable variation in opinion exists as to the importance of strict control of the diabetes in preventing these vascular complications. On the *one hand, there are those who advocate a fairly rigid control with diet* as well as insulin, aiming at as close regulation of the disease as possible, believing that hyperglycemia is harmful. Jackson and his group have been especially emphatic in holding that good control of the young diabetic is both desirable and possible. Wilson, Root, and Marble, reporting on a group of 247 patients whose diabetes began between the ages of eighteen months and thirty years and had been in existence from ten to thirty-four years, have brought forth evidence suggesting that poor control leads to a higher incidence of vascular calcification and retinal lesions than is found in patients under good or excellent control. Similar conclusions were reached by Root, Sinden, and Zanca. However, as noted elsewhere, it is a depressing fact that Wilson *et al.* were able to place only 7 of 247 patients in their "excellent control" group and 30 in the "good control" group. On the other hand is the school which hol[ds] that a liberal regimen *with more or less constant hyperglycemia* and [illegible] but with a daily [illegible] of insulin is more beneficial from the [illegible] al and social p[illegible] [illegible] has not b[een] [illegible] predispose to [illegible] it several m[illegible] young. Im[illegible] diabetics (See [illegible])

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## Chapter 19

# THE PATHOLOGY OF DIABETES IN CASES OF LONG DURATION

Of no little interest are those cases of diabetes which have successfully resisted the disease for a number of years. In this series of 859 cases of diabetes which came to post mortem examination 157 or 18 per cent were of fifteen years duration or longer in contrast to the 300 cases of the first edition in which only 23 or 8 per cent had lived fifteen years with the disease. Now furthermore there are 71 cases of twenty years or longer duration and 26 who passed the twenty five year mark before they died.

There are two factors to be considered in these cases (1) The lo duration of diabetes has given full range for the development of patholog cal lesions secondary to it and (2) there has been ample opportunity fo the degenerative processes of age to complicate the diabetic portion of th picture.

The case of longest duration in this series is No. 511, a male aged seventy years, whose diabetes had developed 39.2 years before death. The immediate cause of death was an acute pleuritis accompanied by vague respiratory symptoms. He was well nourished and well developed. The pancreas weighed 50 grams. The islands were normal in number and showed moderate hyaline change and slight fibrosis. There was marked fatty infiltration of the acinar tissue and marked interlobular and interacinar fibrosis with scattered small retention cysts associated with the ducts. The heart weighed 500 grams, the hypertrophy being chiefly of the left ventricle. There was a healed infarct of the left ventricle and marked myocardial fibrosis. The coronary arteries were tortuous, showed numerous atheromatous plaques and were markedly calcified. The liver was essentially negative. There was a large cholesterol stone in the gall bladder. The kidneys were of moderate size and both showed a moderate degree of vascular scarring with some sclerosis of the large vessels. The aorta showed extreme atheromatous degeneration with marked calcification. There was no gangrene. In other words in this case the diabetic lesions seen were relatively inconspicuous as compared with the lesions which we may be justified in considering as secondary to arteriosclerosis.

Another case of 33.7 years duration, a woman aged eighty four years, died from coronary thrombosis. She weighed 138 pounds after one leg had been amputated at mid thigh. The pancreas weighed only 40 grams. The islands were normal in number and showed a moderate degree of fibrosis. There was a moderate degree of interacinar fibrosis as well. The

heart was moderately enlarged, weighing 440 grams, and showed marked myocardial fibrosis as well as a healed infarct. No fresh infarct had developed from the thrombosis owing to an insufficient lapse of time. The coronaries were markedly atheromatous and calcified and the left was thrombosed. The liver, weighing 1400 grams, was negative. The kidneys were slightly contracted and showed a moderate degree of vascular scarring. The aorta showed marked atheromatous change with calcification. The left leg had been amputated for gangrene. There were many gall stones of the cholesterol type accompanied by a moderate degree of chronic cholecystitis. The eyes showed bilateral cataracts. In this case, just as in the other, while the pancreas showed clear-cut pathology, a considerable number of apparently normal islands were present.

It speaks well for the treatment of the present day that several of these cases have outlived their normal expectancies of life from the age at which they first developed diabetes.

However, by and large, diabetics do not have a normal life expectancy. Millard and Root[1] present an analysis of 110 cases concluding that the average life expectancy fulfilled by these patients was only 44 per cent of normal.

The causes of death in these cases differ strikingly from those of Naunyn[2] in pre-insulin days, over half of whose cases died of coma or tuberculosis. Coma as a cause of death was noted in only 4 of the present group of 157 cases, and 1 of these occurred in the days before insulin. In 3 of these cases the islands were noted as few. The occurrence of definite pancreatic lesions in many of these mild cases of long duration, and the relative absence of these lesions in the severe cases of short duration in the children, is just the reverse of what might be expected in attempting to correlate pathology and symptomatology. A glance at Table 37 shows that one-half of the cases showed some degree of hyalinization or fibrosis of the islands. Three showed evidences of hypertrophy of the islands, 22 showed few islands, and 5 showed probable hydropic change.

Over one-fourth the cases showed no demonstrable pancreatic pathology with the usual routine stains.

Most striking of all is the extraordinary incidence of atherosclerosis, especially of the coronary arteries, as a cause of death. The study of Clawson cited in Chapter 11 showed only about 4 per cent of deaths in a large autopsy series to be directly attributable to coronary sclerosis. In the table above, fully 43 per cent of deaths are directly ascribable to coronary atherosclerosis, and most of the others, of course, had atherosclerosis though it was not listed as the chief cause of death. Vascular disease and infection together account for 85 per cent of the deaths, emphasizing in a most striking manner the two major problems in the management of diabetes.

Table 37 Cases of Diabetes of Fifteen Years Duration or More (1 7 Cases)

| No | Age | Duration | Cause of death | Islands | Aorta arteriosclerosis | Gangrene | Cardiac infarct | Gall stones |
|---|---|---|---|---|---|---|---|---|
| 1 9 | [illegible] | 15 0 | M | | +++ | − | | |
| 33 1 | 54 | 1 0 | M | | M | − | | − |
| 190 1 | 61 | 1 0 | Sep | H+++ | + | + | | |
| 2 09 | 78 | 1 0 | Cardiac infarction | H+++ | +++ | − | Fresh | − |
| 3 879 | 6 | 15 0 | Cerebellar hemorrhage | H+ Few | ++ | − | | + |
| 329 1 | 0 | 15 0 | Coma bronchopneumonia | Few | + | − | | + |
| 81 8 | 43 | 15 0 | Sepsis | | +++ | − | | − |
| 9413 | 64 | 1 0 | Cerebral thrombosis | S++ | +++ | − | | − |
| 40 88 | [illegible] | 15 0 | Pulmonary embolism | H+++ S+ adenoma | +++ | + | Yes | − |
| 43 4 | 4 | 1 0 | Myocardial infarction | | +++ | − | Fresh healed | |
| 464 | 68 | 13 0 | Sepsis | H+ | ++ | − | | |
| 49578 | 60 | 1 0 | Renal insufficiency | H++ | ++ | − | | |
| 3649 | 71 | 15 0 | Coronary occlusion | H+ | +++ | − | Yes | − |
| 981 | 6 | 1 0 | Myocardial infarction | H++ | +++ | − | Fresh healed | − |
| 7 3 | 9 | 1 0 | Anuria secondary to aortic thrombosis | | +++ | − | Yes | − |
| 69317 | 32 | 15 0 | Sepsis | Few small | + | − | Healed | |
| 80 49 | | 1 0 | Operative shock (prednisolone therapy) | Few small | + | − | | |
| 8360 | 63 | 1 0 | Pulmonary embolism | H++ u le o- r a | + | − | | + |
| 83783 | [illegible] | 15 0 | Undetermined | | +++ | + | | − |
| 87877 | 48 | 15 0 | Sepsis | H++ Man la ge | ++ | − | | − |
| 88704 | 2 | 15 0 | Renal insufficiency | S+++ | +++ | + | | − |
| 89800 | 69 | 1 0 | Myocardial infarction | | +++ | − | Yes | − |
| 91970 | [illegible] | 15 0 | Coronary thrombosis | H+ | +++ | − | Healed | + |
| 01790 | 74 | 1 0 | Myocardial infarction | | +++ | − | Fresh healed | |
| 78 | 43 | 1 | [illegible] | | +++ | | | |
| 88 | 6 | 1 3 | Infarct of heart | S+++ | +++ | + | Fresh healed | + |
| 7740 | [illegible] | 15 3 | Cerebral hemorrhage | | + | | Healed | + |
| DA27-4 | 76 | 1 5 | Bronchopneumonia | S+ | +++ | − | | − |
| 7033 | 0 | 1 5 | Septicemia | S++ | +++ | + | Fresh | − |
| 32 78 | 66 | 15 8 | Coronary sclerosis (infarct) | H+++ | + | − | [illegible] | − |
| 87404 | 69 | 1 8 | Rupture of myocardium | | +++ | | Healed fresh with rupture | + |
| 18 | 60 | 16 0 | M | | + | − | | − |
| 31660 | 61 | 16 0 | Myocardial infarction | H+++ | ++ | − | Fresh healed | + |
| 319 4 | 6 | 16 0 | Cerebral arteriosclerosis | H+++ Few | +++ | − | | + |

H—hyalinization
* Post mortem examination limited
S—fibrosis
†—twins
M—information missing

Table 37 Cases of Diabetes ı Iifteen Years Duration or More (I5 Cases)

| No | Age | Du a o | Cause of dea h | Islands | Ao a a ter o- sclero s s | Gan g ene | Card ac infa ct | Ga l s n s |
|---|---|---|---|---|---|---|---|---|
| 1 9 | d | 1 0 | M | | +++ | − | | |
| 33C1 | 54 | 15 0 | M | | M | − | | |
| 19021 | 61 | 1 0 | Seps s | H+++ | + | + | | |
| 27093 | 78 | 15 0 | Card ac nfarc t o | H+++ | +++ | − | F e h | |
| 32879 | 69 | 15 0 | Cerebell r hem o rhage | H+ Few | ++ | − | | + |
| 32909 | 50 | 15 0 | Coma f ron ho pne non | Few | + | − | | + |
| 8178 | 43 | 15 0 | Seps s | | +++ | − | | |
| 9413 | C4 | 1 0 | Cerebral tl rom bosis | S++ | +++ | − | | |
| 40 88 | C | 15 0 | I lmo ary em bol n | H+++ S+ a lenom | +++ | + | Yes | |
| 43 ,4 | 74 | 1 0 | M ocar i al i farct o | | +++ | − | Fresh healed | |
| 4645 | 68 | 15 0 | Seps s | H+ | ++ | − | | |
| 49578 | 60 | 1 0 | Renal ns f fic enc | H++ | ++ | − | | − |
| 3649 | 71 | 15 0 | Co o ar oc l s on | H+ | +++ | − | Yes | − |
| 5C 81 | C | 1 0 | My ocard al n farct o | H++ | +++ | − | Fresh healed | − |
| 57 37 | 9 | 15 0 | An a second ar to aort c th oml os | | +++ | − | Yes | − |
| C9317 | 3 | 15 0 | S ps | Few sm l | + | − | Hea l | |
| 80 49 | 2 | 1 0 | Ope at ve sho k (p elo epl t s) | Few small | + | − | | |
| 83 02 | 43 | 1 0 | I lmo ar em bolus | H++ a eno- n a | + | − | | + |
| 83783 | 6 | 15 0 | U determ ned | | +++ | + | | |
| 87877 | 48 | 15 0 | Seps s | H++ Man large | ++ | − | | − |
| 88704 | 22 | 15 0 | Renal i s f fic e c | S+++ | +++ | + | | − |
| 8 800 | 69 | 1 0 | M ocard al n f rct on | | +++ | − | Yes | − |
| 91970 | 6 | 1 0 | Coronar tl on bos s | H+ | +++ | − | Healed | + |
| 103 9C | 74 | 15 0 | My ocard al farct | | +++ | − | F esh he ed | − |
| 78 | 43 | | I re a l d eas of k d ey | | +++ | − | | − |
| 88 | CC | 1 3 | I f c f lea | S+++ | +++ | + | Fresh h led | + |
| 7740 | 79 | 1 3 | Cerel al he o rl age | | + | | Heal d | + |
| DA27 4 | C | 15 5 | Bron ho e mon | S+ | +++ | − | | |
| 2 033 | 70 | 15 | Sept cem a | S++ H+++ | +++ | + | F esh | − |
| 32 78 | C6 | 15 8 | Coronary s le osis ( f c ) | | + | | Fresh | − |
| 87404 | C) | 15 8 | R pt re of a ora l n | | +++ | − | H aled fresh w h ru tu e | + |
| 18 | 60 | 16 0 | M | | + | − | | − |
| 31C 0 | 61 | 16 0 | My ocard al n fa ct on | H+++ | ++ | − | Fresh healed | + |
| 319 4 | 56 | 1C 0 | Ce ebral ar er oscleros s | H+++ Few | +++ | − | | + |

H h yal n zat o
*—Post mortem examinatio l m ted

S—fib os s
†—twins

M—nfo mation missing

Table 37.—(Continued)

| No | Age | Duration | Cause of death | Islands | Aorta arteriosclerosis | Gangrene | Cardiac infarct | Gall stones |
|---|---|---|---|---|---|---|---|---|
| 10,204 | 44 | 20 0 | Sepsis | Small | +++ | + | | − |
| 17767 | 74 | 20 3 | Sepsis | H++ | +++ | + | | − |
| 6[illegible]8 | 67 | 20 7 | Infarct of heart | H++ S++ | +++ | | Fresh healed | |
| 23442 | 51 | 21 0 | Coronary thrombosis | | ++ | | Healed | |
| 27749* | 60 | 21 0 | Cardiac failure | | M | | M | + |
| 44[illegible]6 | 60 | 21 0 | Coronary occlusion | H+ | +++ | | Fresh healed | |
| 74631 | 30 | 21 0 | Undetermined | | ++ | | | |
| 107070 | 6[illegible] | 21 0 | Cardiac failure (cause?) | Large | ++ | | | + |
| 82,[illegible]5 | 74 | 21 2 | Myocardial infarction | H+ | ++ | | Yes | |
| 3[illegible]72 | 63 | 21 5 | Coronary occlusion | | + | | | |
| 1[illegible]7 | 70 | [illegible] 8 | Coronary insufficiency | S+ H++ | +++ | | | + |
| 2[illegible]0[illegible]7 | 75 | 21 8 | Bronchiectasis | H+++ | +++ | | | |
| 2[illegible] 43 | 50 | 22 0 | Coronary thrombosis | Few | +++ | | Fresh | |
| 280[illegible]8 | 59 | [illegible]2 0 | Acute peritonitis | H+++ | +++ | | | |
| 8[illegible]448 | 67 | 2[illegible] 0 | Diabetic acidosis, clinical (coma) | Few | + | | | |
| 919[illegible]1 | 63 | 2[illegible] 0 | Pulmonary embolism | Small | +++ | | Healed | |
| [illegible]9[illegible] 7 | 50 | 22 0 | Coronary occlusion | Few hydropic change | +++ | | Healed | + |
| 10[illegible]776 | 73 | [illegible] 0 | Intestinal obstruction (cancer) | | ++ | | | |
| 21344 | 66 | [illegible]2 [illegible] | Coronary disease | H++ | +++ | | | + |
| 66 | 72 | 22 2 | Coronary insufficiency | Hydropic change small | +++ | + | | |
| 93312 | 73 | [illegible]2 4 | Pneumonia | Many | +++ | + | | |
| 101[illegible] | 60 | 22 5 | Myocardial infarction | | +++ | | Fresh | + |
| 3[illegible]564 | 68 | [illegible]3 0 | Cachexia (cancer) | H+++ | + | | | |
| 80411 | 59 | 23 0 | Myocardial infarction | Adenoma | +++ | | Yes | |
| 80914 | 84 | 23 0 | Cerebral hemorrhage | | ++ | | | |
| 195301 | 33 | 23 0 | Cerebral embolism (cardiac) | | +++ | + | Yes | |
| 104277 | 30 | 23 0 | Renal insufficiency | Few small | +++ | | Healed | |
| 10516* | 71 | [illegible]3 0 | Sepsis | H++ | ++ | | | |
| [illegible] | 50 | 23 3 | Carbuncle, septicemia | H+++ | ++ | + | | |
| 1[illegible] | 56 | 24 0 | Cerebral hemorrhage | H+ | ++ | + | | |
| 6[illegible] | [illegible] | [illegible]4 0 | Myocardial infarction | S+ Few hydropic change | ++ | | Fresh healed | |
| 85[illegible]3 | 71 | 24 0 | Postoperative state following gastroresection (Cancer) | | ++ | | | |

H Hyalinization S Fibrosis M Information missing
* Post-mortem examination limited + Twins

TABLE 37 —(*Continued*)

| No | Age | Duration | Cause of death | Islands | Aorta arteriosclerosis | Gangrene | Cardiac infarct | Gall stones |
|---|---|---|---|---|---|---|---|---|
| 75794 | 68 | 18.0 | Myocardial infarction | | ++ | – | Fresh | + |
| 79951 | 65 | 18.0 | Myocardial infarction | H+ Few pyknotic nuclei | +++ | – | Fresh | + |
| 80901 | 30 | 18.0 | Myocardial infarction | Many | +++ | + | Fresh | – |
| 93527 | 67 | 18.0 | Undetermined | | +++ | – | | – |
| 96127 | 33 | 18.0 | Renal insufficiency | | +++ | – | | – |
| 26583 | 80 | 18.3 | Coronary occlusion | | +++ | – | | + |
| 27953 | 67 | 18.5 | Coronary occlusion | H+++ S+ Large few | +++ | – | Fresh | |
| 22075 | 57 | 18.6 | Coronary insufficiency | S+ | +++ | | | |
| 20051 | 71 | 19.0 | Sepsis | H+ | + | – | | |
| 3878[illegible] | 44 | 19.0 | Multiple abscesses | S+ Few | + | – | – | |
| 55132 | 75 | 19.0 | Coronary occlusion | Few | +++ | – | | |
| 63825 | 74 | 19.0 | Myocardial infarction | | ++ | – | Yes | – |
| 71381 | 71 | 19.0 | Coronary thrombosis | H+++ | ++ | – | Fresh healed | – |
| 73833 | 72 | 19.0 | Sepsis | | +++ | + | Yes | + |
| 32082 | 40 | 19.0 | Undetermined probably coronary occlusion | S++ | +++ | – | Healed | – |
| 100326 | 73 | 19.0 | Subacute myocarditis | H++ S++ | + | – | | – |
| 86796 | 71 | 19.1 | Rheumatic heart disease | | +++ | – | | – |
| 22533 | 64 | 19.2 | Sepsis | | – | – | | – |
| 24355 | 82 | 19.4 | Undetermined | H+++ Few | +++ | + | | + |
| 17832 | 73 | 19.5 | Rupture of heart | | +++ | – | Fresh with rupture | – |
| 288[illegible] | 63 | 19.7 | Pulmonary embolus | | +++ | – | Healed | – |
| 28129* | 64 | 20.0 | Bronchopneumonia | M | M | – | M | + |
| 28636* | 68 | 20.0 | Pyemia | H+++ | M | – | M | – |
| 15007 | 75 | 20.0 | Acute pericarditis | H+++ S+ | +++ | – | | + |
| 62011 | 52 | 20.0 | Myocardial infarction | H+ | +++ | – | Fresh healed | Organ removed |
| 3330 | 71 | 20.0 | Coronary occlusion | | ++ | – | | – |
| 5[illegible]90 | 80 | 20.0 | Hypostatic pneumonia and cachexia (cancer) | | +++ | – | ? infarct | + |
| 014 | 70 | 20.0 | Myocardial infarction | | +++ | + | Healed | + |
| 73 | 73 | 20.0 | Pulmonary embolism | | ++ | + | | + |
| 18 | 3[illegible] | 20.0 | Pulmonary edema | S+ Few | + | – | | – |
| [illegible] | 70 | 20.0 | Coronary insufficiency | | +++ | – | Healed | M |
| 6 | 45 | 20.0 | Pulmonary insufficiency | | + | – | | – |
| | 71 | 20.0 | Cardiac tamponade | | +++ | – | Fresh rupture | Organ removed |

H—hyalinization
*—Post mortem examination limited
S—fibrosis
†—twins
M—information missing

These findings correlate well with those of Millard and Root[1] who concluded that 85 or 77 per cent of 110 diabetic patients had evidence of atherosclerosis of clinical importance. Likewise, Robbins and Tucker[3] found that in their series of 307 cases, coronary occlusion, peripheral vascular disease, infections of the extremities and acute pyelonephritis were particular hazards for the diabetic patient.

The problem of the possible relationship between this extraordinary incidence of atherosclerosis and the high fat diets which many of these patients had during the early part of their disease is a vexing one, and one which will probably not be answered until years hence when a large number of cases treated on a low-fat regimen become available (see Chapter 9).

It is interesting to compare the table above with the table in the preceding chapter (page 211). It becomes evident at once that *as a cause of death* renal disease is more important in the younger age group, coronary disease in the older. This situation can be interpreted in at least two ways. It could mean (1) simply that the people in the older group lived long enough to develop advanced atherosclerosis, or (2) that the diabetes of young persons differs in severity (perhaps also in kind?) from that of the older group and hence tends to lead earlier to the capillary (and arteriolar) type of vascular damage. It is conceivable, of course, that the older group would eventually have succumbed to the renal type of lesion if atherosclerosis had not been superimposed. These questions do not seem to be resolvable in the present state of our knowledge.

As mentioned in the preceding chapter, the question of the relation of the degree of control of the diabetes to the incidence of vascular complications is a controversial one. Wilson, Root and Marble[4] in a series of 101 patients who had had diabetes more than twenty years found a distinctly higher incidence of vascular calcification and of retinal lesions in those whose diabetes was under poor or fair control than in those under good to excellent control. However, out of their entire series of 247 patients only 7 were able to qualify as having been under excellent control. Dolger[5] on the other hand finds no relation between control of the diabetes and the incidence of vascular disease. (See also the recent summary by Joslin[6].)

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TABLE 37—(Continued)

| No | Age | Duration | Cause of death | Islands | Aorta arteriosclerosis | Gangrene | Cardiac infarct | Gallstones |
|---|---|---|---|---|---|---|---|---|
| 24509 | 74 | 24 3 | Cerebral softening | H+ | +++ | − | | + |
| 9874 | 78 | 2 0 | Infarct of heart | H+++ S++ | +++ | − | Healed | − |
| U23 13 | 62 | 2 0 | Coronary thrombosis | Few | ++ | + | Healed | − |
| M588 | 2 | 25 0 | Septicemia | H+++ | M | − | | − |
| 10 74 | 63 | 2 0 | Circulatory failure (cancer?) | H+++ Many | + | − | | |
| 102948 | 71 | 2 0 | Probably diabetic coma | H+++ adenoma | +++ | − | Healed | − |
| 100910 | 75 | 25 0 | Pulmonary embolism | | +++ | − | | − |
| 7104 | 68 | 25 5 | Streptococcus sepsis | H++ | +++ | + | Fresh | + |
| DA27 57 | 54 | 25 0 | Cerebral hemorrhage | H++ | +++ | − | Fresh, healed | − |
| 3706 | 61 | 26 0 | Septicemia | H++ | +++ | − | | Organ removed |
| 617 7 | 73 | 26 0 | Undetermined, probably cerebral hemorrhage | H+ S+ | +++ | − | Healed | − |
| 10040 | 77 | 26 0 | Coronary insufficiency and myocardial infarction | H+++ S+ Lymphocytic infiltration | +++ | + | Fresh | − |
| 1603 | 63 | 8 1 | Coronary insufficiency | Hypertrophy | +++ | − | Healed | − |
| H15-102 | 38 | 28 0 | Coma | Few | M | M | | M |
| 92028 | 74 | 29 0 | Coronary occlusion | Many | +++ | − | Fresh | + |
| 4996 | 76 | 29 4 | Infarct of heart | Few H+ | +++ | + | Fresh with rupture | + |
| 815 | 77 | 30 0 | Bronchopneumonia | Few H+++ S+++ | +++ | − | | − |
| 23085 | 71 | 30 0 | Mesenteric thrombosis | | ++ | + | | − |
| 97543 | 66 | 30 0 | Coronary insufficiency | Small | +++ | − | | − |
| M890 | 54 | 30 2 | Gangrene and septicemia | H++ | M | + | M | − |
| 104037 | 61 | 32 0 | Undetermined, probably circulatory failure (cancer) | | + | − | | + |
| 80016 | 79 | 33 0 | Coronary thrombosis | H+ S+ | +++ | − | Fresh | − |
| 6365 | 84 | 33 7 | Coronary thrombosis | S++ | +++ | + | Thrombosis healed | + |
| 38 30 | 63 | 3[illegible] 0 | Coronary insufficiency | S+ Few | +++ | + | | − |
| 3804 | 66 | 35 1 | Pulmonary embolism | Hypertrophy | +++ | − | | − |
| 10 [illegible]28 | 87 | 36 0 | Coronary insufficiency | H++ Pyknotic nuclei, few irregular size and shape | +++ | − | | + |
| 511 | 70 | 39 2 | Acute pleuritis | H++ S+ | +++ | − | | + |

H—hyalinization
*—Post-mortem examination limited
S—fibrosis
†—twins
M—information missing

become necrotic, they are desquamated and pass out with the urine. One of the means of diagnosis of the disease is the finding of iron-containing epithelial cells in the urine.

The clinical aspects of the disease are summarized by Marble and Bailey[1] who reported 30 proved cases with skin or liver biopsies. Of these 27 died, the average age at death being 57.8 years and the average duration of the diabetes 4.9 years. Many showed some resistance to insulin (*see also* Chapter 21). These authors emphasize the frequency of hypogonadism as part of the syndrome and describe methods of diagnosis. The recent popularity of needle biopsy of the liver has provided a new and useful

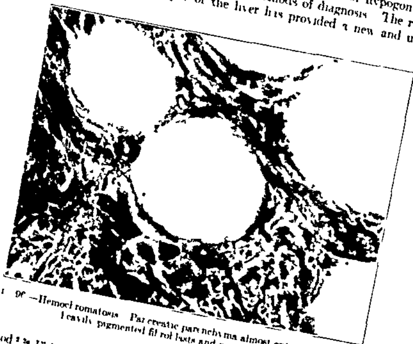


Fig. 96.—Hemochromatosis. Pancreatic parenchyma almost entirely replaced by heavily pigmented fibroblasts and wandering cells.

method.[2,3] Fishback[4] has described a method for demonstrating iron in the skin of the living patient by injecting potassium ferrocyanide and dilute hydrochloric acid directly into the skin.

The initial step in the genesis of hemochromatosis, the formation of the pigment, is as yet not clear. Nevertheless, the effect of the pigment and the correlation of the lesion with the clinical course of the disease are very thoroughly known. In other words, we have in cases of hemochromatosis with diabetic symptoms practically an experimental diabetic condition produced by the action on the pancreas of a known substance. Hemochromatosis without severe involvement of the pancreas does not cause diabetes mellitus. In cases where there is severe pancreatic involvement

except that the course is more rapid and that insulin resistance may be a prominent feature. Here then we have an ideal means of studying diabetes with a known etiology and a fairly *rapid course*.

Bronze diabetes was first described clearly by Hanot.[4] The bronzing of the skin is, of course, not a necessary concomitant of the diabetic syndrome, for it largely depends on how much of the iron has been deposited in the skin. There is not only deposition of iron in the skin but there is *as well an increase of the melanin in the skin causing still further pigmentation*.[2a]

Hanot[4] felt that the diabetes was the underlying factor in the condition. However, Opie[5] pointed out that the "bronze diabetes" of Hanot is identical with the hemochromatosis of von Recklinghausen.[6] Two pigments are involved, the one in which iron can be demonstrated, hemosiderin, and the other in which iron cannot be demonstrated, hemofuscin. Mallory[7] believed that hemosiderin was the product of the intracellular digestion of hemofuscin and that both were derived from the blood pigment.

More recently there is a tendency to doubt that hemofuscin is derived from the breakdown of hemoglobin and to consider it as identical with the "wear and tear" pigment of the older pathologists and as possibly related to melanin.[8] It seems probable that several different pigments are described in the literature under the name of hemofuscin. As indicated by Sheldon[9] and the Gillmans,[10] staining methods may be important.

It is only in the most advanced cases, as a rule, that hemochromatosis produces diabetes. Usually death occurs from hepatic insufficiency before this event or the patient dies from some intercurrent infection. Of 10 cases of hemochromatosis autopsied in Mallory's laboratory none showed sugar in the urine, although they were well developed cases of the disease. The frequent association of exposure to copper with these cases led Mallory to believe that it might be an etiological factor and animal feeding experiments enabled him to produce through the administration of small amounts of copper over a long period of time lesions very similar to those of hemochromatosis. Other workers, however, have not been able to confirm Mallory's observations, and Sheldon,[9] after a careful critical analysis, concluded that there is insufficient evidence to incriminate copper.

At present the etiology must be still regarded as *sub judice*. There is a strong possibility that the disease is due to a defect in iron metabolism, especially since iron retention has been demonstrated in some cases.[11] Sheldon,[9] in an exhaustive survey of the literature and a closely reasoned analysis, concluded, after eliminating various other hypotheses, that hemochromatosis should be considered as due to an "inborn error of metabolism," a theory given support by the occasional occurrence of the disease in siblings (*see also* Rogers[2]). According to this point of view, the storage of iron

continues throughout the life of the individual, not reaching a total quantity sufficient to produce symptoms until middle life, thus explaining the well known age incidence. The marked predominance in males is not elucidated by this theory (unless it be assumed that the menstrual blood loss in the female prevents the disease from becoming manifest).

As noted by Warren and Drake,[11] there has been in the past a tendency to reject the diagnosis of hemochromatosis if a known etiology, as e.g. repeated hemolysis, could be discovered. The morphological basis of differentiation according to Sheldon[9] has been that idiopathic hemochromatosis showed a predominance of hemosiderin in the liver, skin and pancreas, also in the epithelium of the gastrointestinal tract, in the endocrine glands and choroid plexus, while hemosiderosis, as in hemolytic anemias, involved chiefly the reticulo-endothelial system.

However, recent reports of apparently classical hemochromatosis developing in cases where multiple transfusions were given[15, 16] have tended to broaden the general concept of the etiology and pathogenesis of the syndrome (as it should perhaps be called) of hemochromatosis. However, even here the pathogenesis is not as clear as it might be, since frequently evidence of hemolysis is lacking, and, indeed, the amount of iron found may be greater than the calculated amount provided by the transfusions.[6, 18] It is not entirely clear in the present state of knowledge that a distinction can be made between exogenous hemochromatosis and the endogenous disease.[8, 17]

Recently there has also been a tendency to consider dietary deficiencies as playing a major rôle in the development of the disease, a tendency which has been accelerated by the work of the Gillmans[10, 19] in South Africa. These authors, after studying 400 liver biopsies in 120 cases of pellagra, feel that they can state with assurance that hemochromatosis can be regarded as one of the commonest sequelæ of pellagra.[10] They hold that the fundamental defect in hemochromatosis is a profound disturbance in intracellular metabolism induced by dietary imbalance. They believe that both hemosiderin and hemofuscin have a common origin from mitochondria, and have suggested the names "cytosiderin" and "cytolipochrome," respectively, to replace the old and misleading terms.[19] The work of these authors is apparently carefully done and instructive, but it does not seem to justify their sweeping assumption that the pathogenesis of hemochromatosis is thereby settled. The fact that the disease in South African pellagrins shows an equal sex incidence suggests at once that it may be an entirely different disease from classical idiopathic hemochromatosis. Also hemosiderosis does not seem to be characteristic of pellagra as seen in this country.[20, 21] However, the suggestion that a dietary factor may be involved is a useful one and some experimental support may be given this hypothesis by the recent work of Kinney *et al.*,[22] who found that rats on a diet of corn grits absorbed more iron than control animals. These authors also suggested that a low level of dietary phosphate might favor iron storage.

Finch *et al* [24] using radioactive iron found in a case of hemochromatosis that the rate of utilization of radioiron was profoundly depressed but at the same time there was nothing fundamentally wrong with erythrocyte production They conclude (with additional evidence from animal experiments) that radioiron to some extent measures tissue iron stores and that the utilization of it is inversely proportional to the size of these iron stores If this be true the method should prove of great value in the clinical diagnosis of hemochromatosis

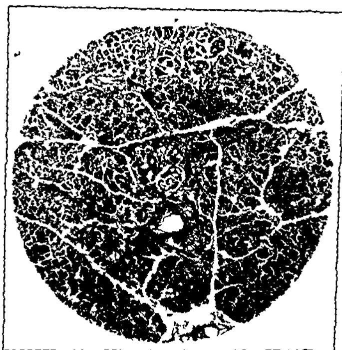


FIG 97 — Hemochromatosis Moderate pigmentation of pancreas Note inter acinar fibrosis also practically normal island in center

*Alper et al* [11] *report that of a total dose of 34 mg of radioiron given* orally to a hemochromatotic patient about 60 per cent was retained The importance if any of ferritin[25] in the metabolic disturbance of hemochromatosis is yet to be settled This substance which is apparently of great importance in the transport and storage of iron is held to be identi[illegible] with the hep[illegible] vasodepressor substance (VDM) of Shorr [illegible]

*of diabetes* simple portal cirrhosis of the liver [illegible] concluded that the three last mentioned diseases all are interrelated They suggest that in hemochromatosis the sequence of events may be as follows

cirrhosis of the liver and diabetes may both be produced by an alloxan like substance, fibrosis of the pancreas then results from portal hypertension consequent to the cirrhosis, and finally hemosiderosis occurs due to abnormal retention of iron derived both exogenously and endogenously. This theory, which is based on several rather tenuous assumptions, still leaves the defect in iron metabolism unexplained.

However, this uncertainty as to the primary etiology in no way interferes with the value of hemochromatosis in throwing light on the pathogenesis of diabetes. In the first place, it is of great importance in showing that

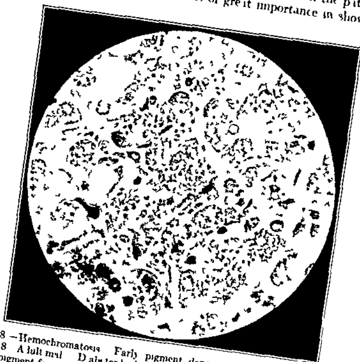


FIG. 98.—Hemochromatosis. Early pigment deposition in island of Langerhans. No. A17-8. Adult male. Diabetes had not developed in this case owing to the presence of many pigment free islands.

perfectly typical diabetes can be produced by a definitely known clinical entity which is known not to be an effect of the diabetic process but to produce it. Moreover, the development of the diabetes is apparently directly related to the extent of the involvement of the islands of Langerhans.

Thus Case L23-31 died of cirrhosis of the liver with ascites, a perfectly typical case of hemochromatosis. There was no diabetes, and while there was a moderate amount of pigmentation of the pancreas, nevertheless, as can be seen from Figure 97, there is practically no involvement of the is-

lands. Many normal islands were seen scattered throughout the section. In contrast to this are Figure 9a and Figure 9b, both from a case of hemochromatosis with heavy pancreatic involvement and with the development of diabetes.

Another case, that of a man aged fifty two years, was of interest owing to his tremendous insulin resistance, and will be described in detail in Chapter 21.

Of the 811 pancreases examined in this series 16 showed marked changes characteristic of hemochromatosis and gave the clinical symptoms of diabetes.

The cases are summarized in Table 38.

*In one case of hemochromatosis without diabetes we found in the pituitary gland a moderate amount of pigmentation. In two additional cases with diabetes pigmentation was fairly marked. In three of the recent cases pigmentation has been remarkably widespread in various tissues throughout the body.*

TABLE 38.—HEMOCHROMATOSIS

| Case No | Sex | Age | *Duration hemochromatosis yrs* | *Duration diabetes mellitus yrs* | *Liver weight grams* | *Pancreas weight grams* | *Skin involved* |
|---|---|---|---|---|---|---|---|
| 12271 | ♂ | 59 | ? | 1 1 | 2170 | 80 | + |
| 14910 | ♀ | 65 | ? | 3+ | 2500 | 45 | + |
| 16343 | ♂ | 53 | 1+ | 0 1 | 2080 | 180 | + |
| 21531 | ♂ | 43 | ? | 0 4 | 2000 | Normal | + |
| 21720 | ♂ | 56 | ? | 2 2 | 1810 | | − |
| 26080 | ♂ | 42 | ? | 0 8 | | | + |
| A22 115 | ♂ | 41 | ? | 0 3 | Small | 90 | + |
| J 6247 | ♂ | 52 | 4 | 1 0 | Large | | − |
| M 2013 | ♂ | 53 | ? | 0 1 | Small | Normal | − |
| 52490 | ♂ | [illegible] | | 1 8 | 1560 | 75 | + |
| 65065 | ♂ | 57 | | 1 5 | 880 | Normal | − |
| 80745 | ♂ | 53 | 0 5 | 7 3 | 2940 | 85 | + |
| 86813 | ♂ | 41 | | 4 1 | 2280 | 90 | − |
| 88667 | ♂ | 42 | 7 8 | 5 0 | 1075 | − | − |
| 10756 | ♂ | 63 | 0 1 yr from diag to time of death | 8 0 | 2600 | About normal | + |
| 10 3 0 | ♀ | 62 | 16 0 | 21 0 | 1500 | 50 | + |

*It must be emphasized that the deposition of pigmentation in the pancreas in hemochromatosis is by no means selective for the islands, but involves the acinar tissues as well. In other words it is not a specific but a generalized process, and the evidence of pancreatic involvement is only of value from the standpoint of the relation of the diabetes to the pancreas rather than evidence in favor of the insular hypothesis itself.*

*Whatever cell is involved, the mechanism is practically the same. According to Mallory,[7] hemofuscin appears first, then hemosiderin. Others doubt that there is any interrelation between the pigments or the hemo-*

fuscin is derived from hemoglobin At any rate both pigments appear Finally the amount of pigment is sufficient to prevent life of the cell When necrosis occurs some of the pigment is taken up by wandering cells some by the stromal cells The stroma condenses new parenchymatous cells are formed and new stroma is laid down only for the process to repeat itself

The hypothesis here suggested that the fibrosis is a direct result of the deposition of the iron pigment is not held by Sheldon [9] who believes that the two processes are independent or by Herbut and Tamaki [28] who point out that the only marked fibrosis is found in liver spleen and pancreas other organs often showing heavy deposits of hemosiderin without fibrosis It is possible however that these differences are essentially quantitative depending on the total amount of iron pigment and the length of time over which it is deposited in a given organ

As Root and Warren[29] have already mentioned in hemochromatosis frequent evidences of attempts at regeneration can be seen considerable numbers of mitotic figures being found in the island cells

Case A17-8 a nondiabetic from Dr Mallory's laboratory is of particular interest in that there were a considerable number of pigment free as well as pigmented islands and in the former islands mitotic figures could occasionally be found

Barker[30] presented a case of much interest in that under insulin therapy which was difficult because of the marked blood sugar variations of the patient swinging easily into hypoglycemia the cutaneous pigmentation diminished

In a series of 30 cases of hemochromatosis diagnosed during life at the Mayo Clinic 26 showed diabetes [31]

One of the most interesting (and unexplained) features of hemochromatosis is the high incidence of primary carcinoma of the liver If one estimates the occurrence by using only the reports in the literature in which 5 or more consecutive cases of hemochromatosis are described one arrives at the remarkably high figure of 18 9 per cent for the incidence of primary carcinoma of the liver in hemochromatosis [11] The reason for the increased incidence over that of carcinoma in simple portal cirrhosis is unknown

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## Chapter 21

# THE PATHOLOGY OF INSULIN RESISTANT CASES

From the theoretical standpoint insulin resistant cases are of great interest though their rarity makes their practical importance slight According to Root [1] a case cannot be considered as falling in this group unless 200 or more units of insulin are needed per day This figure seems to be generally accepted as an arbitrary definition Although originally based on probably erroneous assumptions regarding the insulin output of the normal pancreas it has proved convenient in clinical practice The amount of insulin required in some cases may reach fantastic figures as high as 19 100 units in sixteen hours [2]

The phenomenon of insulin resistance is a curious one which is often unexplained and many apparently conflicting data exist in the literature as is evident from several recent reviews and summaries of the literature [2, 5] The relatively small number of reported cases is probably a very inaccurate index of the incidence of the condition since many cases are unreported [6] In view of the theoretical importance of the phenomenon this paucity of cases is unfortunate all the more so since Haunz[2] states that of 57 reported cases only 8 have come to autopsy

An excellent review of the subject is given by Smelo [3] who points out that although the fundamental mechanism of the hyporesponsiveness is usually not known certain clinical entities are often associated with the condition especially an allergic sensitivity to insulin an endocrinopathy a chronic infection or hepatic disease The only clear cut mechanism of insulin resistance known is the development in some cases of antibodies to insulin This mechanism has been emphasized particularly by Lerman[7] and by Lowell [8, 9] and more recently by Sherman [9] Other authors however tend to minimize its importance For instance Axelrod *et al* [4] found no evidence for an insulin antagonist in their cases by either *in vitro* or *in vivo* methods

On the basis of a careful analysis of 54 cases collected from the English literature Smelo[3] gives the following classification

veloped In August 1927 on a diet of carbohydrate 78 grams protein 60 grams and fat 123 grams he excreted 40 to 90 grams of glucose in t

urine daily and diacetic acid was present although he was receiving 100 units of insulin daily. In September, 1927, he went into coma and required 525 units daily. Insulin was gradually increased until October 22 when he was receiving 840 units per day. October 27, he was given 1600 units and died in coma October 30, 1927, although he had received 1 680 units of insulin in the previous twenty-four hours.

TABLE 39.—CLINICAL STATES ASSOCIATED WITH HYPORESPONSIVE HYPERGLYCEMIA (from Smelo[9])

A Disruption of the functional integrity and innate characteristics of the tissues
  Hemochromatosis
  Recurrent jaundice with common duct obstruction
  Multiple hepatic infarcts
B Dysfunction of enzyme systems
  Lipoatrophy (lipodystrophy) with hepatic cirrhosis
  Defective hepatic glycogenesis
C Dysfunction of hormonal or nervous system regulation
  1 Insufficiency of insulin
    a Delayed absorption
    b Immunologic inactivation or neutralization
      [illegible] tance
      [illegible]
    e Blood dyscrasias with insulin inhibition
      Chronic lymphatic leukemia
  2 Excess of contra insulin hormone
    a Pituitary hyperfunction
    b Acromegaly
    c Cushing's syndrome
    d Hemorrhagic cyst of mid brain
    e Hyperthyroidism
    f Pheochromocytoma
D Pathologic states of etiologic or concomitant significance not present or known

The essential findings in the post mortem examination were a finely granular dark liver and the pancreas showed marked fibrosis and lipomatosis. Microscopic examination revealed deposits of hemofuscin and hemosiderin in the great majority of the organs and very large amounts in the liver and in the pancreas. There was very little pancreatic tissue present most of it being replaced by fat and fibrous tissue. While all of the epithelial elements of the pancreas were pigmented there were a very few islands which were nearly normal. In the adrenals the cells of the zona glomerulosa were extremely heavily infiltrated with the pigments. Presumably but a very slight amount of functioning insular tissue was present in the pancreas and the liver was very severely damaged. There was sufficient liver damage to explain a very considerable degree of interference with the glycogen-storing function. Indeed liver damage would appear to be the only obvious explanation for the well recognized insulin resistance in hemochromatosis.

Another case while it falls short of the true insulin resistant type is nevertheless very instructive (I 24-33) a diabetic woman aged sixty nine years had been a relatively mild case for about ten years. A few months before death there was a marked increase in insulin requirement 50 to 100 units per day being necessary. At autopsy a large carcinoma of the pancreas was found which had destroyed the bulk of the gland. The last 3 cm of the tail were uninvolved and most of the acinar tissue there had

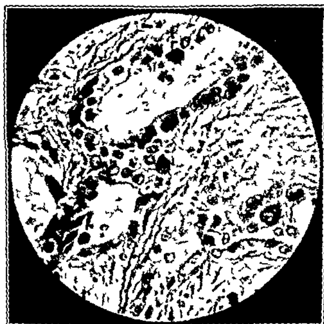


Fig. 93—Carcinoma of pancreas. Very large insulin requirement. No. I 24-33. Female aged sixty nine years. Duration of diabetes sixteen years. Phosphotungstic acid hematoxylin stain. × 250.

atrophied due to duct obstruction while the remaining islands had hypertrophied. Although these islands must have been under considerable functional strain they showed no evidence of hydropic change. Davidson and Eddleman[6] also report a case associated with carcinoma of the pancreas and present a thoughtful review of the literature on insulin resistance. These cases are of interest in relation to the peculiar and unexplained fact that experimental diabetes produced by partial pancreatectomy is often more severe than that produced by total pancreatectomy.[11] One may, of course, speculate on the possible importance of H. G. factor produced by α cells in the surviving part of the pancreas. (*See below* and Chapter 26.)

Root[1] notes that cases with skin lesions may be insulin resistant and suggests that there may be both an allergic factor and a problem of carbo

drate storage However biopsy specimens of skin from nondiabetic persons examined through the courtesy of Dr A M Greenwood show but little variation in glycogen content between normal skin and skin with various pathological conditions

Liver injury[13,14] is one of the most important causes of this phenomenon While liver insufficiency does not in itself produce diabetes it does profoundly influence a coexistent diabetes The importance of the liver for the prompt removal of sugar from the blood is well known and obviously any marked *interference with liver function would lead to lessened glycogen* storage and so decrease the effectiveness of insulin in lowering blood sugar In certain of the nondiabetic cases of cardiac decompensation with liver damage and of cirrhosis of the liver we have found a smaller amount of intracellular glycogen than that usually encountered An instance of sudden insulin resistance *following* thrombosis of the hepatic artery has been reported by Pollack and Long[15]

In marked contrast is a case of portal cirrhosis cited by Bordley[16] A male aged forty nine years was a severe diabetic for five years and then developed jaundice followed by cirrhosis in April 1927 In December 1927 the diabetic symptoms disappeared and in April 1928 and in 1930 he showed no evidence of diabetes mellitus

Interesting observations have been made by Zimmerman *et al*[17] on liver biopsies taken from insulin resistant cases Many of these have shown large amounts of fat The authors do not however regard the fatty livers as directly responsible for the insulin resistance but interpret both the diabetes and the hepatic lesion as due to an unknown causative

cortex have long been
one case improvement
[18] The experimental

diabetes induced in man by pituitary adrenotropic hormone is characteristically *insulin resistant*[19] *Ingle*[20] *in an important review of experimental* work has indicated some of the difficulties involved in any attempt to study factors affecting carbohydrate tolerance Some of his rats treated with adrenal cortical steroids developed tremendous insulin resistance Insulin resistance is characteristically present in the diabetes associated with adrenal tumors *both cortical and medullary* (Chapter 16)

The possible importance of the insulin inactivating system (insulinase) described by Mirsky *et al*[21] in cases of insulin resistance cannot be evaluated as yet This presumed enzyme has been demonstrated in normal rat tissues has been shown to be reduced by fasting and restored by re-feeding but much more work *is required to* assay its significance

One of the peculiar features of insulin resistance is the common tendency *to spontaneous* recovery after a certain period of time About a third of Smelo's[3] cases showed this phenomenon In the great majority *of cases it* cannot be attributed to any therapeutic procedure

The possibility that the hyperglycemic glycogenolytic (H G ) factor presumably produced by the α cells of the islets of Langerhans may play a major part in the pathogenesis of insulin resistance has only recently received consideration in the literature The possibility is mentioned by Lincus[2?] but is elaborated particularly by Lerner [?] who theorizes that the essential difference between insulin-sensitive and insulin resistant diabetes lies in a greater stimulation of α cells in the latter by the diabetogenic factor of the pituitary which he calls the alphacytotropic factor (this assumption being apparently based chiefly on the finding of Ham and Haist[24] that the α cells may be partially degranulated in experimental pituitary diabetes) Such a hypothesis would of course explain conveniently the insulin resistance of experimental diabetes and of the diabetes associated with acromegaly also the otherwise puzzling finding that total pancreatectomy produces a diabetes requiring less insulin than that produced by partial pancreatectomy Though based on little available concrete evidence the hypothesis is an attractive one and should stimulate much investigation in the future Of interest in this connection is the observation that purified growth hormone abolishes the insulin hypersensitivity characteristic of hypophysectomized animals[25]

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## Chapter 22

# HYPERINSULINISM AND THE PATHOLOGICAL EFFECTS OF INSULIN ADMINISTRATION

Hyperinsulinism may be either functional or organic Both types are discussed in excellent summaries of the subject of spontaneous hypogly

following gastroenterostomy in which case the mechanism is apparently rapid "dumping" of sugar upon the absorbing surfaces of the small intestine with a rapid and excessive rise in blood sugar followed by a precipitous fall (due presumably to overstimulation of the $\beta$ cells)

The functional types of hyperinsulinism are of no direct import to the pathologist except as regards possible search for hyperplasia of the islands They are however of great clinical interest since unnecessary surgical explorations of the pancreas have been carried out because of a mistaken diagnosis of adenoma of the islands Factors to be considered in the clinical distinction of the functional and organic types the interpretation of the glucose tolerance test etc are discussed by Conn[1] and by Marble[2] On the other hand Perkins et al [3] emphasize the practical difficulties that may be encountered in individual cases in making a definite diagnosis Conn[1] states that true functional hyperinsulinism is readily controlled by diet (high protein low carbohydrate) designed to prevent the absorptive rise of blood sugar

**Organic Hyperinsulinism** — The case which gave the final proof to the origin of insulin from the islands of Langerhans that of Wilder Allan

importance to warrant presenting a brief summary of it

The case was that of a man aged forty years who had had a gastroenterostomy in 1919 and renal colic in 1924 In January 1925 the fasting blood sugar was normal one specimen of the urine was sugar free while others showed traces of sugar In October 1926 the patient came to the Mayo Clinic complaining of attacks of faintness and weakness for the past eighteen months These were accompanied by profuse perspiration and trembling They usually came on when meals were delayed or if unusual exertion was undertaken The patient found that they could be prevented by eating between meals The first attack resulting in collapse was in

November, 1925. The similarity of it to an insulin reaction led to the diagnosis of spontaneous hyperinsulinism. Exploratory operation was performed December 4, the pancreas was found to be large, hard and nodular, and nodules were present in the right lobe of the liver. The diagnosis of carcinoma of the islands of Langerhans with metastasis to the liver was made. On January 3, 1927, death occurred, apparently from exhaustion. The liver was much enlarged, weighing 3,392 grams. There were five nodules in the liver, ranging from 5 to 0.3 cm. in diameter. The pancreas weighed 120 grams. The tail was thickened and enlarged, and scattered through it were yellowish gray rounded nodules ranging up to 1.3 cm. in diameter. In addition, there was a small cyst at the extreme tail of the pancreas. Microscopic examination showed the cells to be characteristic of those of the islands, as were the nodules in the liver. There was a very large amount of glycogen in the liver cells and in the convoluted tubules of the kidney. Analysis of the liver showed it to contain 8.25 per cent glycogen. Assay of the insulin content of the liver tissue showed that there were approximately 40 units of insulin for each 100 grams of tumor tissue. It is peculiarly interesting that there were large amounts of glycogen in the epithelium of the convoluted tubules of the kidneys in this case of hyperinsulinism.

Closely following this case were the cases of Thalhimer and Murphy[6] in which a large nodule of cells resembling those of the islands was found in the pancreas, apparently a low-grade carcinoma, and of McClenahan and Norris[7] in which a large adenoma of island-cell type had given rise to hypoglycemic symptoms.

The first case of cure of such a lesion was that of Howland *et al.*[8] A married woman, in September, 1922, became semiconscious and restless with sweating and vomiting. At the end of an hour she felt fairly well again. Six of these attacks occurred up to 1924, when they became more frequent and lasted longer, occasionally for twelve hours. Later on she discovered that a glass of milk drunk at the first sign of an attack might ward it off. The physical findings were essentially negative. The blood sugar during one attack dropped to 0.04 per cent. This attack was promptly relieved by the administration of intravenous glucose. On March 15, 1925, operation was performed and an encapsulated nodule 1.5 cm. in diameter, fairly well demarcated, found in the middle of the pancreas. On microscopic examination the tumor was diagnosed as a carcinoma of the islands of Langerhans, owing to the resemblance of the cells to the island type and the presence of scattered mitotic figures. In places the tumor cells directly invaded the surrounding pancreatic tissue. The postoperative course was uneventful and there has been no recurrence of attacks since operation. No evidence of metastasis was noted at the time of operation.[8]

The studies of O'Leary and Womack[9] and Whipple and Frantz[10] have done much to clarify our knowledge of the island-cell tumors.*

* The name "nesidioblastoma" has been proposed for these tumors by Laidlaw[11] and "insulinoma" by Crain and Thorn.[12]

Frantz[12] points out particularly the difficulty of distinguishing benign from malignant tumors especially as regards encapsulation or the lack of it It is possible that many of the

About 20 cases of carcinoma of the islets have been reported most of them without satisfactory differential stains [14 15] The cell type in these tumors is of course of great interest but investigation has been hampered by the lack of adequate techniques for staining the specific granules Laidlaw [1] using Bensley s acid fuchsin methyl green found a predominance of $\beta$ cells in some cases Duff and Murray [16] reviewing the literature found that a varying proportion of cells stained like normal $\beta$ cells in the few cases in which satisfactory stains had been obtained It is probable that some of the newer methods such as the Gros-Schultze silver stain and Gomori s new aldehyde-fuchsin stain (*see* Appendix) will be of great service in the study of these tumors In fact Hess[17] using the Gros-Schultze method has already reported an apparently nonfunctioning carcinoma of the islets consisting largely of $\alpha$ cells

Terbruggen [18 19] using a slight modification of Bensley s acid fuch in methyl green as well as the Gros-Schultze method has demonstrated a few $\alpha$ cells in functional tumors consisting chiefly of $\beta$ cells Terbruggen calls attention to the fact that if one assumes the total weight of islet tissue in the normal pancreas to be about 1 gm as suggested by Ogilvie s work (*see* Chapter 2) then an adenoma weighing only 1 gm would approximately double the insulin producing tissue of the body He also made a careful study of the remaining pancreatic tissue in his cases and arrived at the interesting conclusion that the ratio of $\alpha$ to $\beta$ cells was in the diabetic range *i e* from 1 2 to 1 3 Hultquist [0] also noted a relative increase in silver cells in uninvolved parts of the pancreas from cases of adenoma with hypoglycemia This observation is perhaps to be correlated with Ferner s[2] experimental work on the guinea pig in which he found a reduction in $\beta$ cells and an increase in $\alpha$ cells following repeated injections of insulin (*See* Chapter 2) This finding may likewise be related to the common observation that a period of hyperglycemia lasting several days may follow the removal of an adenoma of the islands

nonfunctioning adenomas using the criteria set up by one of us (S W ) f r the recognition of an adenoma (resemblance in morphology and arrangement of cells to those of normal islands encapsulation with compression of adjacent pancreatic tissue and a diameter of at least 1 mm ) Meissner made the interesting observation that all the functioning tumors contained hyaline similar to that seen in the islands in some cases of diabetes and the nonfunctioning ones did not (with the po sible exception of three ques

tionable cases) The hyaline was even found in the metastases in some of the malignant tumors The relation of this substance to amyloid is unknown but Meissner raises the question of whether it may not be related to the secretion of insulin rather than representing a secondary degenerative change

A remarkably extensive tabulation of no less than 398 recorded cases of tumors of the islands is given by Howard *et al*[21]

While it is generally assumed that diffuse hyperplasia of the islands may be a cause of hyperinsulinism we are not aware of any case in which a completely convincing demonstration of this mechanism has been given Sendrail and Bazex[24] cite two cases of biopsy of the pancreas in which they felt that they could demonstrate an increase of $\beta$ cells without increase in number and diameter of the islands In such cases it would seem that the whole pancreas must be studied and that the considerations relating to total insular mass cited in Chapter 3 should be taken into account Relief of symptoms in a patient following subtotal pancreatectomy is of course no reason for assuming the existence of hyperplasia Careful differential counts of $\alpha$ and $\beta$ cells correlated with estimations of total volume of the islands would however be of great interest

## PATHOLOGICAL EFFECTS OF INSULIN ADMINISTRATION

An abundant literature[2] has grown up which deals thoroughly with the insulin reaction and hypoglycemia A wide range of symptoms has been reported even to transient attacks of hemiplegia[25] Little however is known as to the demonstrable anatomical changes

Fortunately there are but two deaths from overdosage of insulin in the present series

Wohlwill[6] has described 2 fatal cases in which the brain was examined after death The first was a woman aged sixty two years whose diabetes had been present two years A breast abscess developed For three days before death which occurred in coma she had received 100 units of insulin daily Her urine was sugar free and there was no acidosis

The second case a diabetic aged twenty nine years whose disease was of one and one quarter years duration was admitted in coma Insulin to the amount of 135 units was given the first day 70 units the second and 90 the third when death occurred The blood sugar dropped from 0 462 to 0 065 per cent in twenty four hours

Sections of the brain in each case showed a marked amoeboid appearance of the glia cells The ganglion cells showed degenerative changes with pyknotic nuclei and loss of normal staining reaction and in the first case the axis cylinders were markedly swollen and irregular

Cerebral changes have been reported[7] in a case of a woman aged forty nine years whose diabetes had been present for five years The patient experienced several insulin reactions after having been on protamine

insulin four months She was found in hypoglycemic coma and remained comatose in spite of treatment until she died eight days later At autopsy but little island tissue was found The brain was edematous with extravasations of blood about the smaller vessels Much cerebral arteriosclerosis was present

Wolf's[3] case showed no edema of the brain but minor loss of cortical cells and mild ependymitis Terplan[29] noted in addition to marked edema of the brain and cord extreme capillary injection and marked destruction of nerve cells especially in the third and fifth layers

Baker and Lufkin[30] ascribe the ganglion cell changes to post mortem alterations and emphasize numerous new and old hemorrhages throughout the brain which they believe are most numerous in those patients with the most severe convulsive seizures

Cerebral changes were apparent in Case No 12420 an eight year old boy who died from hyperinsulinism He had had diabetes for four years Edema of the brain was marked even to the extent of subependymal blebs and vascular congestion was striking Case No 32878 a boy aged sixteen years suffering from pulmonary tuberculosis and diabetes of five years duration had the misfortune to have while in a sanatorium a hypoglycemic attack which was diagnosed as coma Five hundred units of insulin had been given before the mistake was recognized and glucose was then given in a vain attempt to avert death The liver contained much glycogen the brain was edematous with capillary congestion and some loss of cerebral cortical cells

Lawrence *et al*[3] found extensive nerve cell degeneration and glial proliferation in 6 fatal cases

changes[3, 35]

w found

w it first

showed liquefaction and later pericellular incrustation and finally disappeared rman

regarded of the

lesions anges

produced by thujone camphor and metrazol

Chesler and Himwich[37] studying insulin hypoglycemia in the dog noted a progressive *decrease in brain glycogen occurring* (with the exception of the cerebral cortex) from the rostral part caudad

Wicks[38] points out that the pattern of destructive lesions of the brain

such simple formula as the richness of blood su

other factors especially the individual metabolic patterns of different brain

be important for instance the fact that the consumption

Hicks found that in rats given large doses of insulin the cerebral cortex and corpus striatum were damaged, sometimes severely, but the corpus callosum, substantia nigra and cerebellum were never involved. The neurons of the fascia dentata as well as the pyramidal layer of the hippocampus were extensively necrotic in several animals. The thalamus showed rare necrotic lesions in some instances. There was no strict correlation of dosage and brain damage but some of the most severe lesions followed single bouts of hypoglycemia.

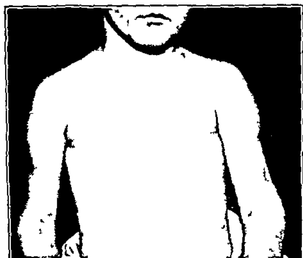


Fig. 100.—Boy five years of age. Duration of diabetes 1.8 years. Note bilateral atrophy of subcutaneous tissue in regions where insulin had been injected. General condition and nutrition good.

While the changes ascribed to hyperinsulinism, or better to extreme hypoglycemia, are not very characteristic, there seems to be ample justification in making the diagnosis of "changes compatible with hyperinsulinism." When combined with these there is definite clinical evidence of hyperinsulinism with chemical evidence of hypoglycemia, the diagnosis may be definitely established. From histological findings alone such a diagnosis is not justifiable.

However, tremendous amounts of insulin may be given without apparent ill effect in certain cases. A striking example is Joslin's Case No. 6247 reported by Root[23] and cited in Chapter 21 as an example of insulin resistance, in which 1,680 units daily *failed to prevent the development of coma* and no changes referable to the insulin were found post mortem.

Occasionally local changes are noted in the skin at the site of insulin injections. In the first few months of insulin production painful nodules

were rarely noted in the skin of injected patients developing soon after injection and persisting for some weeks. These were apparently due to the type of antiseptic then in use and are never seen at present.

*Adlersberg*[10] *has reported multiple skin nodules considered to be lipomas* developing in a girl twenty years of age who had been injecting insulin six years.

*Insulin Lipodystrophy*.—Localized atrophy of the subcutaneous fat is due to repeated injections of insulin in the same area. Barborka[41] and Depisch[4] have reported several cases. The depressed character of the lesions, which range from 2 to 9 cm in diameter and are about 1 cm in depth, and the absence of any evidence of inflammation make their appearance very characteristic. Occasionally these foci may be painful. Isaac[43] suggests a small amount of lipolytic ferment may be mixed with the insulin in such instances. This fails to explain most cases. Trauma to fat is the most likely explanation. Recovery may occur.

The experimental observations of Renold *et al.*[44] may have some bearing on this phenomenon. These investigators found that insulin would increase the deposition of glycogen in fat cells to a significantly greater extent in diabetic rats than in normal animals and that insulin had a direct local effect on the adipose tissue at the site of injection, causing the greatest increase in glycogen in this region.

**Hypersensitivity.**—Hypersensitiveness to insulin was noted[45, 46] not infrequently in the early years of its use before the foreign proteins had been adequately removed. These reactions usually took the form of serum sickness, although an occasional case of true anaphylaxis has been reported. Insulin allergy was noted in 10 per cent of patients at the Mayo Clinic.[47] Gastrointestinal hypersensitivity has been noted.[48]

The clinical importance of hypersensitiveness to insulin is extremely [illegible] e symptomatology [illegible] the patient. For

While insulin hypoglycemia may have a damaging effect on the heart hampered by coronary disease, perhaps through a stimulation of adrenal [illegible] ent altera[illegible] cks.[35]

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## Chapter 23

# INFANTS OF DIABETIC MOTHERS

It has been known for many years that there is a high incidence of stillbirths and of neonatal deaths in the pregnancies of diabetic women [1-9] This holds true for both the preinsulin and insulin eras, although there has been a striking improvement in recent years The causes are obscure and post-mortem examination of the infants has done little to shed light on the problem Certain interesting findings may, however, be noted and recorded particularly the resemblance of these infants to those suffering from erythroblastosis fetalis,[10] and the influence of the diabetic and prediabetic state on the size of the baby [11-15] and on certain tissue changes

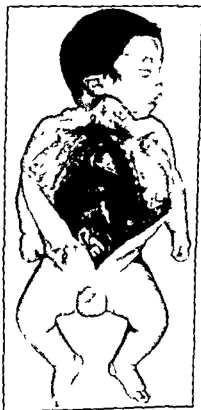


Fig 101 —Typical post-n ... quately treated) The plum ... ceromegaly are well shown

The information on which this chapter is based is largely derived from a study of 50 autopsies on infants of diabetic mothers under the care of Drs. Priscilla White and Raymond Titus, performed at the Faulkner Hospital* from 1937 to 1949, inclusive.

In the latter part of this period the majority of the mothers had been maintained on the hormonal regimen described by White.[3-6] Of the 50 infants, 25 were delivered by Cesarean section.



Fig. 102. Hyaline membrane ("vernix membrane") lining alveoli in lung of infant of diabetic mother.

Table 40. Causes of Death

| | |
|---|---|
| Undetermined (including 11 macerated stillbirths) | 18 |
| Prematurity (under 32 weeks or 1500 gm.) | 13 |
| Aspiration of amniotic fluid | 8 |
| Birth injury | 4 |
| Pneumonia | 3 |
| Congenital anomalies | 3 |
| Adrenal hemorrhage | 1 |
| | 50 |

**Causes of Death.** In the accompanying table (Table 40) are listed the causes of death in the 50 cases. A few words of explanation are in order since the causes listed in the table are, in many instances, not the same as

* Practically all of these necropsies were done by or under the direction of Dr. J. B. Hazard, Dr. G. K. Mallory, or Dr. P. M. LeCompte.

those recorded in the protocols For instance a number of cases originally signed out as pulmonary atelectasis or prematurity are here classed as undetermined since pulmonary atelectasis *per se* was not considered a satisfactory cause of death and prematurity was arbitrarily defined as noted below The undetermined classification also comprises all of the 11 stillbirths in the series since no adequate cause of death could be assigned to any of them (evidence of intrauterine anoxia was not convincing) Prematurity was arbitrarily defined as present whenever a gestational age of thirty two weeks or less or a birth weight of 1500 gm or less was recorded Death was attributed rightly or wrongly, in 8 cases to aspiration of amniotic fluid the chief criterion being the presence in all of a well marked vernix membrane in the lungs (*See* Fig 102 and discussion below ) The birth injuries were, in all instances meningeal tears The anomalies which were sufficient to cause death in three instances consisted of 2 congenital cardiac malformations and one cephalo meningocele *

**Body Weight and Body Length** Macrosomia is perhaps the most commonly noted finding in the infants of diabetic mothers Kraus[21] gives the following tabulation of weights of 608 infants of 155 diabetic women

| | |
|---|---|
| Normal children | 385 (63 32%) |
| 4 Kg children | 110 (18 10%) |
| 4½ Kg children | 47 (7 73%) |
| 5 Kg children | 49 } (10 85%) |
| 5 to 6 Kg children | 17 } |

The large size of these infants has been emphasized particularly by Miller who has also called attention to the fact that apparently normal women who have excessively large babies are likely to develop diabetes in later life [1 11 12 13] This extremely interesting complication of the prediabetic state has been confirmed by others [2 3 24]

The *observed weight* in 43 cases was compared with the *calculated weight* according to the recorded gestational age ** These calculations were based on the empirical formulæ of Scammon and Calkins[25 26 27] which are presented in graphic form by Dunham [20] and which compare fairly well with the data of Yllpo [28] (These formulæ are for fetal *dead* weight which according to Scammon and Calkins [25] averages about 200 gm less than the live weight ) Of the 43 babies only 10 were less than the calculated weight and most of these were under 1500 gm Of 30 infants weighing over 2000 gm at birth only 2 were less than the calculated weight Three infants exceeded the calculated weight by less than

*

**

400 gm 14 were from 400 to 1000 gm heavier and 16 or more than a third exceeded the calculated weight by over 1000 gm

Similarly the *observed length* was compared with the calculated length [2] Of 40 infants for whom data were available 13 were less than the calculated length one was the same two were from 0 to 2 cm longer five were from 2 to 3 cm longer and 18 were over 3 cm longer Five babies were heavier but shorter than the calculated standards and two were lighter but longer (both of these however were under 1000 gm )

**Prematurity** —One striking fact which emerges from the data is that the relatively large size of most of these babies is extremely deceptive and that to judge from the menstrual history the great majority of these babies (at least in this series) must be regarded as premature Standards for judging prematurity are of course arbitrary but commonly accepted upper limits are thirty-eight weeks of gestation 2500 gm body weight and 47 cm body length [20] In the present series 42 of 47 babies were at less than thirty-eight weeks of gestation when born according to the menstrual history and on the same basis the *calculated* weight of 38 out of 42 would be less than 2500 gm and the *calculated* length in 34 of 40 would be less than 47 cm Admittedly this conclusion necessitates placing considerable reliance on the menstrual history but as stated above this is thought to

centers are not available (*See below*)

**Heart** —Among the organs the heart occupies a position of major importance because of previous observations particularly by Miller [29-31] that these infants may show a considerable cardiac enlargement In trying to determine what may be considered a normal heart weight for the newborn and especially for prematures we encountered the same difficulties recorded by Miller [3] as regards variations in published data After inspection of a number of such figures it was decided that the formula devised by Miller [31] would be satisfactory especially since his calculations seem to give if anything a slightly lower figure than some others Miller's formula is as follows

$$y = 0.00624x \pm 0.63 + \sigma\ 3.27$$

where y is the weight of the heart and x the body weight in grams

Application of this formula to the present series yielded the following results of 41 infants of diabetic mothers 19 (or 44 per cent) had hearts which exceeded the calculated weight by more than twice the standard deviation Out of a control group of 70 infants only 6 (or about 9 per cent) had hearts exceeding the expected value by more than twice the standard deviation It seems likely then that nearly half of the infants in the present series had significant cardiac enlargement It is of interest that 2 of

3 infants with erythroblastosis fetalis in the control group had heart weights exceeding the expected figure by more than twice the standard deviation

Considerable amounts of *glycogen* have been demonstrated in the hearts of infants of diabetic mothers chiefly ~~by the rather unreliable~~ Best carmine staining technique[10, 37] and the hearts of diabetics in general appear to contain more glycogen than normal[33] (*see* Chapter 5) A marked vacuolation of the cardiac muscle fibers seen best in cross section is a common finding in these infants often appearing more striking than that of the normal fetal heart which also contains a good deal of glycogen

Since it seemed possible that the glycogen content might account for at least some of the apparent enlargement and might also account for some of the circulatory and respiratory difficulty observed in these babies chemical analysis for glycogen was carried out (courtesy of Dr Albert Renold) on five hearts from infants of diabetic mothers and one heart from an infant of a nondiabetic mother

TABLE 41 —GLYCOGEN CONTENT OF INFANT HEART

| Aut No | Hrs post mortem | Heart | Liver | Muscle |
|---|---|---|---|---|
| A 49 1 | 4 | 3 52% | 4 86% | 3 32% |
| A 49 13 | 3 | 0 26% | | |
| A 49 20 | 7½ | 0 024% | | |
| A 49 23 | 5½ | 0 188% | | |
| A 50-33 | | 1 38% | | |
| A 50 36 (nondiabetic) | | 0 14% | | |

Each heart was frozen solid shortly after removal from the body and kept so until the analysis was carried out The results are shown in Table 41 It is recognized of course that figures such as these are of dubious value in view of the time that elapses between death and necropsy due to the necessity for obtaining autopsy permission *etc* Cruickshank[33] gives average figures of 0 60 and 1 25 per cent for normal and diabetic hearts respectively according to this two of the hearts would be distinctly in the diabetic group

The reasons for the remarkably high content in A 49 1 exceeding the values in some cases of glycogen storage disease[34] are not at hand At least one infant of a diabetic mother has been reported as an example of glycogen storage disease[35]

**Lungs** —The gross and histologic findings in the lungs do not appear to differ essentially in most cases from the picture in normal controls Atelectasis of varying degree was present in every case but the lung structure resembled that seen in control cases of stillbirths and neonatal deaths No instances of alveolar dysplasia as described by MacMahon[36] were seen The amount of amniotic fluid in the lungs seemed greater in many cases than in comparable controls In 8 instances of neonatal death (not still births) the presence of a thick pink-staining hyaline layer often giving a

faintly positive Sudan stain ("vernix membrane") plastered on the lining of the occasional open alveoli (Fig 102) seemed sufficient for a cause of death. It is hard to conceive of much diffusion of gases through such a liver[37] and the membrane itself must tend to increase the cohesiveness of the alveolar surfaces and thus prevent neighboring alveoli from opening. Although most of these cases were delivered by Cesarean section one of the most severe was a spontaneous delivery, hence it seems unlikely (although it is still possible)[38, 39, 39a] that the mode of delivery is a major factor in promoting aspiration of amniotic fluid.

The postulation of this cause of death is admittedly an assumption and a guess. It seems to be generally agreed that there is a tidal flow of amniotic fluid in the larger air passages of the fetus *in utero* and that more or less vigorous respiratory movements may occur before birth[4 ... 4]. Certainly traces of amniotic fluid in the form of cornified epithelial cells may be found histologically in the lungs of practically any stillborn or newborn infant. However, a well marked "vernix membrane" can hardly be neglected as a possible cause of respiratory embarrassment. The application

suction[5] to the new

the hyaline membrane does not represent vernix or the result of aspiration of amniotic fluid but rather a reaction to injury analogous to the membrane seen in adults in the pneumonia associated with pandemic influenza or in so-called atypical pneumonia. They found the membrane almost confined to premature infants dying within forty eight hours of birth and coming from mothers whose pregnancy and delivery were uneventful. More recently Miller *et al*[46] have modified this view and apparently hold that the membrane is a manifestation of pulmonary edema which they think may be related to dysfunction of the vagus nerves since they have produced something similar in animals by section of the vagi. In both animal and human material they believe they have demonstrated a polysaccharide in the hyaline membrane.

Dick and Pund[47] believe the membrane to be formed of vernix but suggest that the vernix must have a high fat content; in this way they explain the absence of the membrane in some cases in which it would be expected to be present.

Bruns and Shields[48] hold with Miller that the hyaline membrane is not due to aspiration of amniotic fluid. They point out the variety of conditions in which such a membrane has been observed both in human and experimental material and emphasize the possibly important fact that a similar membrane can be produced experimentally by high concentrations of oxygen, thus raising the disturbing question of whether overzealous inhalation therapy may not predispose to formation of the membrane.

Blystad *et al*[39a] on the basis of 500 autopsies and some experimental work hold that the membrane is composed of the concentrated protein of

amniotic fluid that it is most common in premature infants and in more mature infants with a history of a complication such as Cesarean section and that it is a major cause of death in the neonatal period

Obviously much more work must be done on this crucial question

**Spleen** —The weights of the spleens were compared with the expected figures for the observed body weight using the table of Potter and Adair[6] which gives weights for various organs in terms of body weight In 4[illegible] cases the following results were obtained in 19 cases the observed weight of the spleen was less than the expected weight in 7 cases it was the same

[illegible]

questioned whether the figures are significant in view of the wide variations in organ weights that may be encountered[49] It seems possible that if any increase of weight were present it might be accounted for on the basis of islands of erythropoiesis which in some cases seemed more abundant than would be expected for normal infants of the same gestational age

**Liver** — Again the figures of Potter and Adair[16] were used as standards In 42 cases in which adequate figures were available the results were 11 were from 0 to 25 gm over the expected weight 15 were more than 25 gm over the expected figure and 8 of these were more than 40 gm overweight In the control group of 50 cases 21 were from 0 to 25 gm over the expected figure 7

40 gm [illegible]

infants [illegible]

these cases since the great majority were premature infants (*see above*)

[illegible]

pancreas by eosinophilic leukocytes

Hyperplasia of the islets was *considered to be present in 23 of 27 cases In a control group of 20 cases hyperplasia was found in 5 cases (It is note*worthy that 2 of these 5 were cases of erythroblastosis fetalis further discussion of this point *follows below*) *Since actual measurement of islets* requires serial sections of the glands conclusions as to presence or absence of hyperplasia were based on simple inspection combined with measurements made with a micrometer eyepiece The *great majority of the cases* regarded as hyperplastic exhibited islands which exceeded the 250 micra in diameter suggested by Potter *et al*[60] as the approximate upper limit of normal

number [illegible]

larged hyperchromatic nuclei [illegible]

In many instances the hyperplasia was so extensive that entire low power microscopic fields composed solely of islands of Langerhans could be found (Fig 103) Frequently the hyperplastic islets contained enlarged hyperchromatic nuclei In a few cases there was striking evidence of the formation of islets from small ducts the ducts sometimes being incorporated within the islet (Fig 104) The great majority of the cells making up the large islets seem to be $\beta$ cells as could be shown in a few favorable cases with Gomori's chrome alum hematoxylin stain and as Hultquist *et al* [51] have shown with the Gross-Schultze stain The $\alpha$ cells appear to be present in approximately normal numbers the increase in size of islets being due exclusively or almost so to proliferation of $\beta$ cells

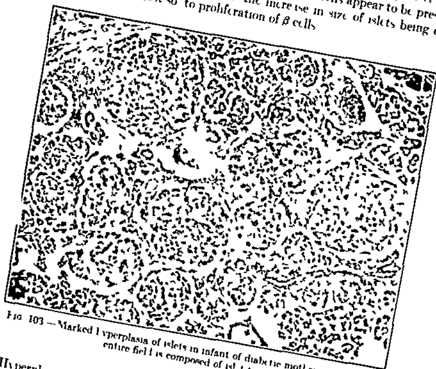


Fig 103 —Marked hyperplasia of islets in infant of diabetic mother Almost the entire field is composed of islet tissue

Hyperplasia of the islets of Langerhans in the infants of diabetic mothers has been recognized for some years [19 60–69] However it does not occur in all such infants nor is it specific for maternal diabetes as Potter *et al* have pointed out [40] The cause of the hyperplasia has usually been considered to be the maternal hyperglycemia with consequently increased demands for insulin Indeed it has been shown that the severity of the mother's diabetes may be reduced during pregnancy only to return to its former state after delivery thus suggesting that the insulin of the fetus is being used by the mother [40 60] Also experimental work suggests that certain



Fig 104 —Hyperplastic island of Langerhans in infant of diabetic mother Small ducts are included within the island, and some acinar tissue is intimately associated with one end Some nuclei are large and hyperchromatic

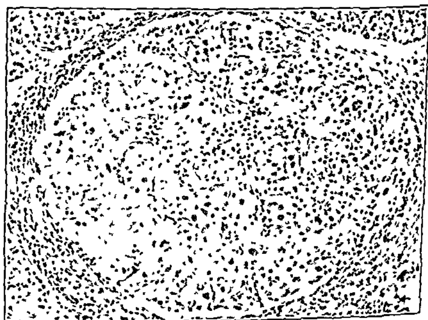


Fig 105 —Hyperplastic island of Langerhans from infant of diabetic mother The leukocytes within and around the island are eosinophils

adult animals, e g rat[11] are capable of hyperplasia of the islets in response to hyperglycemia and that the young of other species may have the same ability.[6] Evidence against hyperglycemia as the stimulating factor in islet hyperplasia is however embodied in Miller's[11,12] claim that the infants of prediabetic women with normal blood sugars may show the same change.[11]

Of great interest is the previously described hyperplasia of the islands of Langerhans which occurs in some (not all) cases of erythroblastosis fetalis.[13,14] No plausible explanation of this phenomenon seems to have been offered.

A striking change which may be found in the pancreases of infants of diabetic mothers is an intense infiltration of eosinophilic leukocytes. These are found usually in the interstitial interlobular tissue but are often intra-acinar and not infrequently are concentrated around and within the hyperplastic islets (Fig 105). More or less eosinophilic infiltration was noted in 12 cases. This phenomenon has been noted by others[10,15] and was described in the last edition of this book as representing extramedullary hematopoiesis. It may well be such although the presence of large masses of eosinophils and particularly of what appear to be mature eosinophilic leukocytes in some cases suggests an infiltration or exudate. In other instances the cells resembled eosinophilic myelocytes more suggestive of extramedullary hematopoiesis especially since similar cells are often found elsewhere particularly in the interstitial tissue of the thymus in both the present series and in normal control infants.

**Gastrointestinal Tract.**—Nothing of note was found in this system.

**Adrenals.**—Particular attention was paid to the adrenal glands in view of current interest in their relation to carbohydrate metabolism and in view of the fact that they have been stated to be enlarged in the infants of diabetic mothers.

It soon became apparent that any attempt to form judgments concerning the weight of these glands was futile. Not only were the published normal values for the weight of these glands (Scammon[16,17] Cruickshank[18]) found to vary tremendously but when Scammon's formula for the weight of these glands was applied to this and a control series the results shown in Table 42 were found.

TABLE 42. WEIGHTS OF ADRENAL GLANDS IN INFANTS

| | *Less than calculated* | *Same as calculated* | *Overweight but less than 3 × S D* | *Over 3 × S D* | *Total* |
|---|---|---|---|---|---|
| Diabetic mothers | 12 | 1 | 5 | 12 | 30 |
| Controls | 11 | 2 | 8 | 18 | 42 |

In almost half of both the diabetic and control series the adrenals exceeded the calculated weight by more than three times the standard deviation. It seems obvious that no conclusions can be drawn.

17

A series of about ten adrenals from infants of diabetic mothers and ten control cases were studied in frozen section by some of the histochemical techniques in current use for the demonstration of ketosteroids (i e staining with Sudan III and examination of sections treated with acetone and of untreated sections by polarized light) No significant differences between the two series were noted There appeared to be no difference in the x zone or androgenic zone [67 68 69] in those infants who had lived more than a few hours the expected involutionary changes[70] were found to be beginning in this region in both groups

The general lack of distinctive morphological findings in the adrenal appears to correlate well with the results of eosinophil counts done on about a dozen of these infants at the Faulkner Hospital [71] Counts were done as soon as possible (usually ten to twenty minutes) after delivery by Cesarean section also at four hours one two three five and seven days after delivery The data when compared with a small control group of Cesarean sections on nondiabetic mothers showed no apparent significant differences Infants of both groups were usually born with relatively high counts (around 600 or more per cu mm) then showed a sharp drop (usually less than 50 per cent however) at four hours then a maximal fall to around 100 to 200 sometimes less at twenty-four hours followed by a rise the next day and more or less stabilization at a level distinctly lower than that found at birth

**Kidneys** —Kidney weights were compared with calculated normals based on the figures of Potter and Adair [16] Of a series of 39 cases 16 were less than the calculated weight 9 were the same (i e within 1 gm) and 14 were over the calculated value Only two instances of marked enlargement (i e more than 30 per cent over the expected weight) were encountered and in these cases the accuracy of the figures may be questioned No significant trend was apparent

Glycogen was demonstrated apparently in the loops of Henle by the Best carmine method in two cases in which the maternal blood sugar had been high just before delivery (Fig 106) This observation would seem to be further evidence for a high fetal blood sugar reflecting maternal hyperglycemia and would also seem to reinforce the hypothesis that so called glycogen nephrosis is simply the result of an elevated blood sugar Hinde[73] reports glycogen in the collecting tubules in the normal newborn of several species of mammals including man However in the few normal infants that we have examined with this point in mind the glycogen has not appeared to be so sharply localized as in the infants of diabetic mothers

Potter and Thierstein[74] have indicated their belief that the structure of the fetal glomerulus is a valuable criterion for estimating the age of the fetus After a study of the kidneys of 1000 infants they concluded that immature glomeruli were rarely seen over thirty five weeks of gestation in those weighing over 25 in those measuring more ... cm in length In general at glomerular maturity

) in loops of Henle in kidney of ... Best carmine stain × 12)

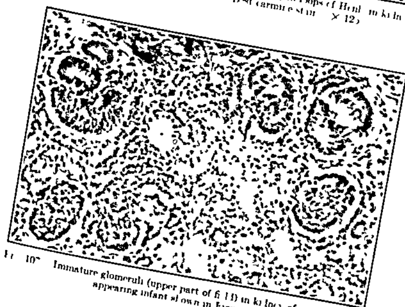


Fig. 107. Immature glomeruli (upper part of fig. 11) in kidneys of postmature appearing infant shown in Figure 101

seemed to be more closely related to the size of the fetus than to its gestational age. In a few cases, however, the reverse was true.

In Table 43 are listed the data on 4 large infants in this series in whom immature glomeruli were found. Their gestational age was around thirty-

TABLE 43.—LARGE INFANTS SHOWING IMMATURE GLOMERULI

| *Autopsy No.* | *Gestational age, weeks* | *Weight gm.* | *Length cm.* |
|---|---|---|---|
| A 48-58 | 36 | 4200 | 51 |
| A 49-1 | 34 | 3750 | 51 |
| A 49-13 | 35 | 3080 | 49 |
| A 49-15 | 34 | 3460 | 50 |

five weeks, but all weighed well over 2500 gm. and measured 49 cm. or more in length. (*See also* Fig. 107.) If the conclusions of Potter and Thierstein are correct, these data provide further evidence for regarding some of the infants of diabetic mothers as large prematures rather than postmature infants. Others[75] also feel that these infants should be treated as prematures.

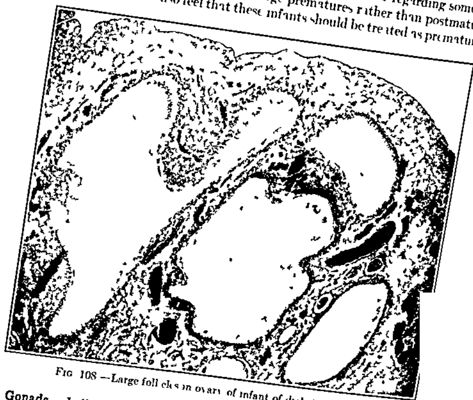


FIG. 108.—Large follicles in ovary of infant of diabetic mother.

**Gonads.**—Follicular cysts lined by granulosa cells and surrounded by theca lutein cells have been described[68, 76] in infants of diabetic mothers and have been attributed[76] to the high concentration of chorionic gonadotropin

which is said to occur during part of the gestation period in these women [5 77 78 79]

Follicles with granulosa cells and theca lutein cells were found in 3 of 10 female infants in this series (Fig 108) and in at least one of these no hormone therapy had been given to the mother In one infant a striking decidual reaction of the endometrium was encountered (Fig 109) In this case however no follicle cysts or corpora lutea were found in the ovaries The mother had received large doses of estrogen and progesterone The possible effect on the fetal gonads of the large doses of hormones which



Fig 109 —Decidual reaction in stroma of endometrium Infant of diabetic mother × 250

many of these women received is difficult to assay especially since so little is known on the subject [80 81 82] In two male infants vacuolization of the prostatic epithelium possibly attributable to massive doses of estrogens given to the mother was observed Such a finding is not however reported by Davis and Potter [82]

The presence of Graafian follicles in the ovary must be interpreted with caution since in many cases the infants of nondiabetic women may show them [83] The incidence in the diabetic series does appear to be higher however An alternative explanation for their presence may be found in the work of Abel [84] This investigator showed that large doses of insulin in

adult female mice led to marked follicular development and eventually to the appearance of numerous corpora lutea If it be granted that the hyperplasia of the islets of Langerhans in these babies reflects an overproduction of insulin a possible effect on the ovaries may be considered although Abel apparently obtained no definite results in immature animals

**Other Endocrines** — No changes considered significant were found in the thyroid parathyroid or pituitary glands In view of the technical problems and the scarcity of data on normal values for infants no attempt was made to count acidophil and basophil cells in the pituitary In some instances the acidophil cells did show an apparent predominance as noted by others [85 86] Some difficulty was encountered in judging the number of basophils since in some cases both basophils and chromophobes seemed to take a similar dull blue color with Mallory s aniline blue stain

**Thymus and Lymphoid Tissue** —In histological examination of the lymphoid tissues in newborn infants it is common to find more or less shrinkage of the lymphocytes with pyknosis and fragmentation of their nuclei This change is usually most obvious (and apparently appears first) in the medulla of the thymus but when well advanced may be found in the cortex of the thymus spleen lymph nodes and other foci of lymphoid tissue as in the intestines

Particular attention was paid to this phenomenon in the present series in view of recent work on disintegration of lymphocytes by adrenal cortical steroids [87] since it was considered that such an appearance might reflect the presence of an intrauterine alarm reaction [88] in these infants It was found however that no such conclusion could be drawn Shrinkage of lymphocytes was invariably absent in the stillborn infants and was commonly present in those who lived more than a few hours being most extensive as a rule in those who survived several days Comparable results were obtained in the control series It thus appears probable that the disintegration of lymphocytes whatever its cause is a phenomenon of extrauterine life

**Bones** No distinct differences between diabetic and control series were noted in sections of bone (usually rib or vertebra) No adequate roentgenological investigation of density ossification centers etc has been carried out

**Placenta** —The condition of the placenta was of particular interest because of the fact that premature aging of this organ has been postulated[17] as the cause of the sharp drop in urinary chorionic gonadotropin observed in the latter part of pregnancy in diabetic women A series of 54 placentas ... ed

series of 53 placentas from nondiabetic mothers

No definite differences in gross appearance between the two series were made out However when the weights were compared calculating the amount of placenta per kilogram of baby weight the diabetic placentas

were found to be definitely heavier, the mean for this group being 195.5 gm. per kg. baby weight as opposed to 165.5 gm. for the control group. [illegible]

this figure. A few of the relatively heavy placentas belonged to quite premature infants, weight 3 to 5 pounds at birth, but the majority belonged to infants weighing 6 to 8 pounds.

The two series were not strictly comparable in other respects: for instance, half of the diabetics were delivered by Cesarean section (hence may have had slightly more blood in the placenta), while practically all the controls were normal spontaneous deliveries. Nonetheless, there seems to be a significant difference between the two series. Perhaps the heavier placenta in the diabetics may prove to be another manifestation of immaturity (*see below*).

It would seem that the sharp drop in chorionic gonadotropin observed in diabetic women[77] might be reflected in a difference in lipoid content between the diabetic and normal placenta. Accordingly, we have applied some of the methods of Wislocki and Bennett[89] and Dempsey and Wislocki[90] (chiefly Sudan staining and polarized light) to several diabetic placentas and several normal ones. No definite differences could be made out between the two groups. Others[75] have found no characteristic changes in the diabetic placenta.

However, McKay[91] reports, in a series of these placentas fixed within a few minutes after delivery by Cesarean section, the finding of a definite stromal edema of the larger villi. This was quantitatively quite variable, sometimes involving the majority of the villi, sometimes only a few. This edema disappeared when the placentas stood at icebox temperature for a few hours. McKay suggests that the edema may be simply a manifestation of immaturity of the placenta, but this is not yet established. Another change noted occasionally in these edematous villi was the presence of mitotic figures in cells of the cytotrophoblast, which was quite prominent in some of the large villi. Preliminary histochemical results indicate that ketosteroids are absent from the trophoblast of these placentas.[*]

**Congenital Anomalies.**—It has been generally stated that congenital anomalies are more common in infants of diabetic mothers.[1,3] This appeared to be true in the present series, in which 9 of the 50 infants presented congenital defects, summarized in Table 44.

TABLE 44.—CONGENITAL DEFECTS IN 50 INFANTS OF DIABETIC MOTHERS

| | |
|---|---|
| Cardiac | 6 |
| Cephalo-meningocele | 1 |
| Cystic kidney | 1 |
| Absence of one kidney | 1 |

One of the infants with a cardiac anomaly also had multiple skeletal defects. It is interesting that the commonest anomaly was a small interventricular septal defect, this being the sole anomaly in 4 of the 6 cardiac cases. Congenital defects were considered to be the cause of death in 3 cases (2 cardiac and 1 cephalo meningocele).

**Experimental.**—The experimental investigation of the problem of fetal mortality in diabetic pregnancies has been fraught with difficulty. Diabetes induced by alloxan in pregnant animals of various species[92, 93] has been shown to result in a high incidence of intrauterine fetal death and of abortion, but oversized fetuses have apparently not been produced by means of alloxan. Hultquist,[94, 95] however, in a careful study using partial pancreatectomy during pregnancy, was able to obtain giant fetuses in rats. These giant fetuses showed hyperplasia of most of the endocrine glands (thyroid and ovaries were not enlarged, and there was only questionable hyperplasia of the islets of Langerhans). The pituitary glands of these large fetuses were said to show increased vascularity and enlarged cells with *prominent nucleoli, basophils seeming to predominate.* In further experiments Hultquist and Engfeldt[96] were able to produce giant rat fetuses by administration of various hormones, particularly growth hormone, to the mothers during pregnancy. The changes in the endocrine glands of these fetuses were said to be similar to those previously obtained when the mothers were subjected to partial pancreatectomy. The authors emphasize the fact that these giant offspring were obtained without prolongation of pregnancy, in contrast to certain other experiments which they cite where large postmature fetuses were obtained by artificial prolongation of pregnancy. The mechanism by which growth hormone may act to produce giant fetuses is still to be elucidated. Large fetuses were also obtained, but to a lesser extent, when chorionic gonadotropin or thyrotropic hormone was given.

**Summary.**—The cause of death in the majority of the infants of this series, even those who were not early prematures, is unfortunately not clear, and the reason for the high intrauterine and neonatal death rate remains obscure.

It seems clear, however, that as a group the infants of diabetic mothers present certain characteristic findings, most of which may be expected to be present in babies born in the latter part of gestation, but some or all of which may be absent, particularly in early prematures. These findings are as follows:

(1) *Macrosomia.*—It is a clear-cut and widely recognized fact that the babies of diabetic mothers tend to be larger than normal. The tendency for this large size to obscure actual prematurity has been mentioned above. It seems quite as justifiable and probably much more useful to regard these babies as large prematures as to call them postmature. The evidence provided by glomerular immaturity noted above may perhaps be cited as positive evidence in this regard. The common tendency to use size alone

as a criterion of maturity[97] is certainly open to question Excellent discussions of the difficulties of establishing a diagnosis of postmaturity are given by Ballantyne and Browne[98 99]

The hypothesis that the large bodily size of the infant is due to overactivity of the maternal pituitary a particularly attractive theory in relation to the prediabetic state is not as yet supported by adequate evidence

(2) *Visceromegaly* —Enlargement of the heart is seen frequently enough to be considered significant Although it is probable that these hearts contain more glycogen than normal ones the increased size can hardly be due to the glycogen deposit unless one assumes that glycogen may make up more than 10 per cent of the weight of the heart Enlargement of the spleen and liver is seen in only a fraction of cases and is not clearly significant It may be due in part to relatively large amounts of hematopoietic tissue although it is not clear that the latter is actually increased out of proportion to the age of the fetus (*see below*)

(3) *Pancreas* —Two changes are found often enough to be considered significant, (a) hyperplasia of the islets of Langerhans and (b) infiltration of eosinophilic leukocytes The causes of both of these phenomena are obscure

(4) *Hematopoiesis* —Extramedullary hematopoiesis (chiefly erythropoiesis) especially in the liver and spleen has been cited as characteristic of these infants As noted above the significance of this finding is questionable when one considers the gestational age of the fetus If it is truly increased the cause is not clear

(5) *Glycogen Nephrosis* —Deposition of glycogen in the renal tubules may be expected when there has been an unusually high maternal blood sugar shortly before delivery However as noted above the specificity of this finding in the newborn may be questioned

(6) *Gonads* —Growth of follicles in the ovary may be found in a certain percentage of cases and may conceivably be due to a high concentration of chorionic gonadotropin or possibly to an excess of insulin from the hyperplastic fetal islets of Langerhans

(7) *Placenta* —In this series many of the placentas seemed to be definitely heavier than normal Whether this is related to immaturity remains to be seen No changes considered to be significant were found in the ad[illegible]

and spleen hyperplasia of islets of Langerhans and hematopoiesis) has been noted and provides interesting opportunity for speculation If it could be shown that anoxia stimulates the islets to hyperplasia a possible common ground for the two conditions might be found As yet no explanation for the similarity is at hand However as noted by Miller[10 11] caution must be exercised to avoid confusing the two especially when diag

nosis is based on pathological examination alone in the absence of adequate information concerning the mother

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# Chapter 24

## CANCER AND DIABETES

A RELATIONSHIP between cancer and diabetes has long been suspected but has been difficult to establish on sound statistical grounds. Such an association was mentioned but not elaborated upon by Wilson and Maher[1]. Marble[2] analyzed 256 cases in which the two diseases were associated. These represented the cases of cancer recognized among 10 000 cases of diabetes, i.e., an incidence of 2.56 per cent. Marble also studied the figures of insurance companies and other data and concluded that while certain evidence suggested that cancer was more common among diabetics, such a conclusion could be questioned.

Drs and Lessmer[4] reviewed 182 cases of diabetes and found 38 cases of cancer, a remarkably high incidence of 20.9 per cent. It is of interest that 6 of these 38 cases had carcinoma of the pancreas.

Ellinger and Landsman[4] in studying 1,280 cases of diabetes found 39 cases of cancer, i.e., an incidence of 3.04 per cent. This they found to correlate well with an incidence of 2.95 per cent in 14 332 cases of diabetes from the world's literature. They compared these figures with an incidence of cancer for the general population of 0.46 per cent (figures for New York State in 1941.)

Apparently the most careful statistical study of the problem to date is that of Jacobson.[5] He notes the importance of avoiding Wilson's[6] fallacy of operational selection and of studying the universe of the living as well as the universe of the dead. He also points out that it is not sufficient to show that a given condition is less frequently associated with another at the time of death; rather it must be shown that the first condition is less frequently associated with the second than it is with other diseases and conditions. He demonstrates that cancer is as frequently associated with diabetes as it is with other conditions (e.g., syphilis, tuberculosis) that are generally thought to be associated with cancer, and that cancer is more frequently associated with diabetes than it is with other conditions (e.g., cardiovascular renal disease) that are not thought to be associated with cancer. Jacobson concludes, applying Wilson's principle, that cancer and diabetes were reported as joint causes of death in New Jersey in 1940 and in New York City in 1930 more frequently than would be expected if these two causes fell independently upon the total population. His general conclusion is that the incidence of cancer is higher among diabetics than among nondiabetic individuals.

The incidence of cancer as a cause of death in the present series was 10 per cent (81 of 818 cases—see Appendix C). This figure is doubtless in part attributable to the fact that the New England Deaconess Hospital is recognized as a center for the treatment of cancer as well as diabetes. Of some interest is the distribution of types of cancer according to tissue and organ systems in diabetes. In Table 45 are presented the sites of origin of 132 cases of cancer occurring in diabetics. Although few conclusions



Fig. 110.—Adenomatous post partum hyperplasia of endometrium in diabetic woman treated during pregnancy with estrogens. × 125

can be drawn from such a small group, it seems apparent that the incidence of carcinoma of the endometrium, carcinoma of the pancreas, and pheochromocytoma of the adrenal is higher than would be expected in a group of nondiabetics of similar size.

In a series of patients with carcinoma of the endometrium, Moss[7] noticed a rather high incidence of diabetics. He thought that this was probably to be expected in view of the obesity and age of patients suffering from this type of cancer. Meissner and Sommers[8] have studied the post-partum endometrial hyperplasia found in some 50 diabetic women who were

curetted from three months to two years after delivery These women had been maintained during pregnancy on Dr Priscilla White's regimen of large doses of stilbestrol and progesterone and these hormones were presumably responsible at least in part for the findings which consisted of endometrial hyperplasia often polypoid sometimes cystic sometimes adenomatous sometimes a combination of both * This hyperplasia differed from the ordinary type in involving a more regular and more florid overgrowth of both glands and stroma and in being quantitatively more marked In typical cases the glands were closely packed tortuous adenomatous and dilated with abundant compact cellular stroma often containing eosinophils (Fig 110) The authors point out that in some series of endometrial carcinoma from 9 to 11 per cent of the patients are diabetics[7,10]

Of interest in this connection is a study by Sommers[10a] as yet incomplete of a series of multiple malignancies in which a small group of women has been found showing various morphologic stigmata of hyperpituitarism These women tend to have diabetes mellitus and have multiple cancers involving the endometrium ovaries breast and gastro-intestinal tract in various combinations

Of considerable interest is the 14 per cent incidence of carcinoma of the pancreas in Table 45 This stands in contrast to figures of from 2 5 to 4 8 per cent for the incidence of carcinoma of the pancreas in the general population [1] The question of a possible etiological relationship at once arises and with this in view the 19 cases are listed in Table 46 in an attempt to compare the duration of the cancer and of the diabetes It is evident that in over half the cases the symptoms of carcinoma either antedated the symptoms of diabetes or occurred apparently simultaneously with them This time relation does not of course necessarily indicate a causal relation but certainly makes it more likely

In this connection it may be noted that Dashiell and Palmer[1] in a series of cases of carcinoma of the pancreas found glycosuria (usually inconstant) in 27 per cent and a diabetic type of glucose tolerance curve in 18 of 21 patients They suggest that the value of this test in the diagnosis of carcinoma of the pancreas may be greater than has been realized Levy and Lichtman[3] made similar observations However as noted by Marble[*] cancer patients of any type are poor subjects for the glucose tolerance test and it would seem that all such observations should be carefully controlled

Silver and Lubliner[11] found that 14 of 43 cases of carcinoma of the body or tail of the pancreas had diabetes while only 2 of 57 cases of carcinoma of the head of the pancreas had diabetes One might of course suspect that this was true because carcinoma of the head of the organ is likely to produce biliary obstruction and death before enough of the pancreas is destroyed to bring out the diabetic syndrome

* In a more recent series of pregnant diabetic women this complication has not been noted presumably because of the use of a different oil vehicle for administration of the hormones *

TABLE 45.—CANCER COMPLICATING DIABETES

| | *Male* | *Female* | *Total* |
|---|---|---|---|
| Breast | 0 | 12 | 12 |
| Lung | 1 | 2 | 3 |
| Alimentary tract | 0 | 1 | 1 |
| Lip | 1 | 0 | 1 |
| Buccal mucosa | 1 | 0 | 1 |
| Pharynx | 1 | 0 | 1 |
| Tongue | 1 | 0 | 1 |
| Stomach | 1 | 3 | [illegible] |
| Colon | 8 | 8 | 16 |
| Rectum | 8 | 2 | 10 |
| Pancreas | 12 | 7 | 19 |
| Liver | 4 | 0 | 4 |
| Gall bladder and bile ducts | 0 | 3 | 3 |
| Adrenals (pheochromocytoma) | 3 | 0 | 3 |
| Kidney | 1 | 2 | 3 |
| Bladder | 4 | 2 | 6 |
| Prostate | 12 | 0 | 12 |
| Uterus (endometrium) | 0 | 9 | 9 |
| Cervix | 0 | 2 | 2 |
| Vagina | 0 | 1 | 1 |
| Ovary | 0 | 2 | 2 |
| Leukemia | 1 | 2 | 3 |
| Malignant lymphoma (Hodgkin's) | 1 | 1 | 2 |
| Thyroid | 1 | 2 | [illegible] |
| Skin | 2 | 2 | 4 |
| Miscellaneous | 1 | 2 | 3 |
| Totals | 67 | [illegible] | 132 |

TABLE 46.—DURATION OF DIABETES IN RELATION TO CARCINOMA OF THE PANCREAS

| *Path No* | *Duration of diabetes* | *Duration of carcinoma* | |
|---|---|---|---|
| DA 26 37 | 3 3 yr | ? 2 yr | ? Simultaneous |
| 55364 | 1 mo about | About 4 mo | Simultaneous |
| 10830 | 1 2 yr | 1 yr | |
| 12850 | 5 mo | Less than 1 yr (1 2 mo) | Simultaneous |
| 13372 | 10 yr | 3 yr | Antedates |
| 16295 | 6 mo | 1 yr | |
| 19061 | 1 5 yr | 1 yr | ? Simultaneous |
| 22683 | 3 mo | Less than 1 yr (1 2 mo) | ? Simultaneous |
| 29273 | 10 2 yr | 1 yr | |
| 32832 | 4 yr | Not stated | |
| 57232 | 5 wk | 1 mo | Simultaneous |
| 60311 | 1 mo | 1 yr | Antedates |
| 68600 | 8 yr | 8 mo | |
| [illegible] | 12 yr | Less than 1 yr | |
| 81320 | 1 5 yr | Not stated | |
| 82595 | 21 yr | Not stated | |
| 84772 | ? 6 wk | Discovered at admission (1 wk) | ? Simultaneous |
| 86702 | 4 yr | 3 yr | ? Simultaneous |
| 102682 | 13 mo | 1 yr 2 wk | Simultaneous |

The strikingly high incidence of primary carcinoma of the liver in hemochromatosis is unexplained The subject is reviewed by Warren and Drake[15] (see also Chapter 7)

The important subject of tumors of the endocrine glands in relation to diabetes is discussed elsewhere (see especially Chapter 16)

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Chapter 25

# EXPERIMENTAL DIABETES

In the following discussion, emphasis will be placed on the pathogenesis and histopathology of experimental diabetes. For further details and more complete bibliography, several excellent reviews may be consulted, especially those of Lukens,[1,2] Houssay,[3,4,5,6] Bailey,[7] Duff,[8] Bell,[9] Haist,[10] Lazarow,[11] Young,[12] Bennett and Evans,[13] and Jores.[14] None of these by itself constitutes a complete survey of the field, but all taken together provide fairly adequate coverage of the various subdivisions.

In the light of present knowledge, it may be stated that all types of *permanent* experimental diabetes, *i.e.*, all types in which the diabetic state is irreversible and persists after discontinuance of the exciting agent, depend upon destruction of the $\beta$ cells of the islets of Langerhans in the pancreas. In all cases, therefore, there is insufficiency of the endocrine part of the pancreas which is incapable of secreting the amount of insulin required by the organism. Even when the diabetes is initiated by extrapancreatic factors (as, *e.g.*, in pituitary diabetes) the development of a permanent diabetic state is accompanied by, and dependent upon, demonstrable lesions of the $\beta$ cells of the islets. The pancreas is, of course, not the only organ which takes part; the presence of the liver, for instance, is essential for the production of a diabetic state; damage to the pancreas is, however, the *sine qua non*.

Numerous methods are available for producing a temporary glycosuria with or without hyperglycemia, *e.g.*, alimentary, by stuffing the organism with excess carbohydrate; various drugs, such as epinephrine; hormones, such as some of the adrenal cortical steroids; interference with the nervous system, as in the *piqure* experiment of Claude Bernard; and phlorhizin, which has the peculiar effect of rendering the kidney tubules incapable of reabsorbing sugar, thereby leading eventually to increased ketogenesis and lowered R.Q. Some of these types of temporary diabetes will be mentioned again at the end of the chapter.

There are five principal methods for producing a permanent diabetes:

1) Total or subtotal pancreatectomy.
2) Injection of alloxan, or similar substances (including dehydroascorbic acid).
3) Administration of anterior pituitary extract.
4) Thyroid feeding in the partly depancreatized animal.
5) Administration of excessive amounts of glucose over a prolonged period.*

* At the present writing, the diabetic state produced by ACTH or adrenal cortical steroids has not been shown to be permanent. It is therefore omitted from this list and discussed at the end of the chapter.

**Pancreatectomy** —Ever since the classical experiments of von Mering and Minkowski[15] it has been recognized that total removal of the pancreas results in diabetes Since total pancreatectomy also leads to various undesirable side effects subtotal pancreatectomy has been more extensively used Removal of the greater part of the pancreas (nine-tenths in the dog five-sixths in the cat 95 per cent in the rat) usually leads to a mild diabetes which may become permanent In the dog subtotal pancreatectomy does not produce diabetes if as much as one-seventh to one-eighth of the pancreas is left in provided that insulin treatment is given during the first week after operation and that aseptic technique is followed When a *larger amount of the gland is removed* there frequently occurs a mild diabetes (Sandmeyer s diabetes) which is not stable may either disappear spontaneously or increase in severity and lead to death of the animal *This mild diabetes (and in fact any type of experimental diabetes)* is made worse by high carbohydrate diet in some instances a dog with a subtotal pancreatectomy and a normal blood sugar may be made diabetic by *feeding sugar In any case the diabetes does not remain on a plateau* as regards severity it either disappears or gets worse An interesting feature of subtotal pancreatectomy in the rat is the fact that diabetes does not appear immediately but only after a lapse of two or three months Houssay[4] points out that this period is equivalent to several years in the life span of man and suggests that a subclinical prediabetic state without symptoms may precede the appearance of human diabetes

The lesions in the islets after subtotal pancreatectomy were first clearly described in dogs by Allen[6 17] and in dogs and cats by Homans[18 19 20] aided by the then new staining techniques of Lane and of Bensley They noted within a few days after the operation diminution in the granules of the $\beta$ cells accompanied or followed soon by swelling of the cytoplasm and the appearance of clear vacuoles which contained nothing stainable by the methods which are usually used to demonstrate fat glycogen or other substances For a time the cell membranes remained intact eventually however rupture and disintegration occurred followed finally by disappearance of the $\beta$ cells During this process the $\alpha$ cells preserved their integrity and no leukocytic exudate was observed The vacuolated appearance of the $\beta$ cells in the early phase is termed hydropic change and is seen in other types of experimental diabetes *Vacuolation of the epithelial* cells of the small ducts of the pancreas may also be seen (As noted in Chapter 3 the recent work of Toreson[21] indicates that hydropic change in some if not in all instances actually represents glycogen deposition )

The rapidity of development of these lesions was found by Allen to be dependent on the severity of the diabetes In severe unchecked diabetes the earliest vacuoles appeared in about five days *marked or maximal* vacuolation in three weeks and after six weeks to two months all $\beta$ cells had usually disappeared

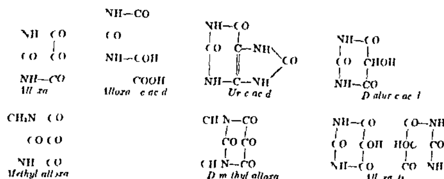
The question of reversibility of the hydropic change is an interesting one. Allen[17] found that the stage of maximal vacuolation usually lasted for about five days. During this period he tried unsuccessfully to abolish the lesions by fasting the animals. Later, however, with the advent of insulin, it was clearly shown by others[20] that reversal of the hydropic change could be achieved by treatment with insulin. This phenomenon becomes more understandable if it is indeed true that the "hydropic" appearance is due to glycogen.[21]

The pathogenesis of the lesions in the islets is a problem to which Allen devoted much attention. He found no evidence that nervous or circulatory factors were concerned. He noted that the severity and rapidity of appearance of the changes varied directly with the severity of the diabetes, that a high carbohydrate diet facilitated their development, and that they could be prevented by fasting the animal or giving a pure fat diet. He found, however, that production of an intense hyperglycemia over a period of three to thirty-eight hours did not produce vacuolation in nondiabetic animals, nor did it lead to perceptible increase in vacuolation in animals already diabetic. Also, in his hands, phlorhizin did not prevent the appearance of hydropic change after pancreatectomy even though the blood sugar was kept at or below normal levels. Also, phlorhizin glycosuria did not produce vacuolation in nondiabetic animals. Allen therefore concluded that the hydropic change was not dependent on nervous or circulatory factors and not due to hyperglycemia or glycosuria. He postulated a functional overstrain of the islets due to some unknown humoral agent. As will be seen below, more recent evidence suggests that hyperglycemia *per se* (or else some factor or factors constantly associated with hyperglycemia) may be [illegible] actually due to [illegible] Yet it seems [illegible] this glycogen deposition with insulin even in the presence of hyperglycemia.[22]

Total pancreatectomy, of course, exerts its effect by sudden and complete removal of the insulin producing $\beta$ cells of the islets. Subtotal pancreatectomy removes most of the $\beta$ cells and may lead to secondary damage of those that survive. It is a fact that the diabetes due to subtotal pancreatectomy (or alloxan) commonly requires for its control more insulin than that due to total pancreatectomy. This finding is discussed in the next chapter.

**Alloxan.** In 1943 Dunn, Sheehan and McLetchie[24] reported that intravenous injection of alloxan into rabbits produced an initial hyperglycemia followed by hypoglycemia and death, accompanied by necrosis of the islets of Langerhans in the pancreas. This announcement was soon followed by the almost simultaneous reports of Brunschwig *et al.*[25] and Bailey and Bailey[26] that permanent diabetes could be produced by this method.

Alloxan is the ureide of mesoxalic acid. Its structural formula and those of some related compounds are shown in the accompanying diagram.



Of these compounds all are diabetogenic except alloxanic acid uric acid and dimethyl alloxan One of the essentials in determining diabetogenic activity appears to be the presence of an intact pyrimidine ring Activity is abolished by substitution of the alloxan molecule at any position other than in one imino group but even in this site if the substitution is carried beyond a certain length of side chain (e g butyl alloxan) the compound becomes inactive It is probable that the diabetogenic effect of certain compounds related to alloxan e g diethylalloxantin dialuric and methyl dialuric acids depends on their being rapidly oxidized in the blood stream to the corresponding alloxans [27]

Recently Lukens and Kennedy [8] have shown that a styryl quinoline derivative will produce necrosis of $\beta$ cells comparable to the effect of alloxan

Alloxan in proper dosage (which varies in different species) has produced permanent diabetes in the rabbit rat hamster dog cat sheep monkey pigeon and man Other species e g guinea pig toad owl are resistant for reasons unknown [7] The resistance of the guinea pig may be due to its high blood glutathione [29]

The typical response of the blood sugar following the intravenous injection of alloxan is represented by a triphasic curve (1) a phase of hyperglycemia of unknown cause appearing two to four hours after injection (2) a hypoglycemic phase at six to twelve hours possibly due to blockage of glycogenolysis in the liver [30,3] and (3) a permanent hyperglycemia ensuing at eighteen to twenty four hours In some animals the resulting diabetes

tests for insulin tolerance or sensitivity resemble those seen in depancreatized animals The severity of the diabetes may be modified by hypophysectomy [3] adrenalectomy [33] thyroidectomy or feeding thyroid extract [34]

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clamping the pancreatic vessels for five minutes during and after the injection prevents the necrosis of the islets.[36] Clamping the renal or splenic pedicle has also a preventive effect, perhaps due to a vasomotor reflex affecting the pancreas.[37] The action of alloxan may also be prevented by the injection of various substances, *e.g.*, glutathione, cysteine, BAL, and many others, provided that they are injected either immediately before, simultaneously with, or within a few minutes after the injection of alloxan.[8, 11, 31, 33] One of the interesting side effects of alloxan is the marked fall in blood glutathione which follows its injection.[24] Alloxan added to plasma is converted almost entirely to alloxanic acid.[40] This stable conversion product may provide a valuable means of studying the relation, if any, of alloxan to human diabetes.

The histopathology of the islets in alloxan diabetes has been studied most carefully in rabbits, rats, and dogs and has been particularly well described by Bailey, Bailey, and Hagan[41] and by Hughes, Ware, and Young.[4] The earliest changes are discernible as early as five minutes after injection of a diabetogenic dose of alloxan and consist in a slight diminution in number of granules in the β cells, with slightly increased prominence of the nuclear membrane and some irregularity of the cell outline. After ten to fifteen minutes there is a definite reduction of granules, affecting first the β cells in the centers of the larger islets. At one hour there is evidence

appearance with homogeneous eosinophilic cytoplasm. Thereafter disintegration of both cytoplasm and nuclei becomes more apparent, with coalescence of cells and evidence of karyolysis. By the end of twenty-four hours extensive disintegration and disappearance of cells is the rule, the centers of the islets usually containing only amorphous granular debris. Only after about forty-eight hours is there a marked fall in insulin content of the pancreas.[42] After several days the granular material is removed by an unknown mechanism and the islet may consist only of a peripheral layer of α cells. The latter cells are spared, according to most reports, although some writers describe complete disappearance of islets, and some report increase in number of α cells. Eventually one may find shrunken islets made up only of these cells (Plate III). In other instances a few β cells may survive. The fate of the D cells in alloxan diabetes is not known with certainty, probably because most authors have not used the specialized and rather difficult techniques necessary to demonstrate them. (*See also* p. 21.)

Throughout the entire sequence there is a striking absence of the leukocytic infiltration usually associated with necrosis of tissue in other well-recognized pathological processes. Also, the lack of eventual fibrosis or hyalinization of the islets is an interesting feature, although this may per-

haps be explained as due to the rather specific action of alloxan on the β cells without associated damage to the connective tissue stroma

Hydropic change of the β cells which is so characteristic of other types of experimental diabetes as noted elsewhere in this chapter is a late phenomenon in alloxan diabetes It does not appear in the early sequence (described above) which follows the injection of a single diabetogenic dose of alloxan probably because the injury to the cells is so acute and severe Occasionally hydropic change may be observed after several weeks in animals made diabetic in this way and has been attributed by Kennedy and Lukens[44] to the effect of hyperglycemia on β cells which escaped destruction by the alloxan Bailey *et al*[41] noted hydropic change al o mitotic figures in some of the islets of animals given repeated small doses of alloxan Toreson[21] has demonstrated the presence of glycogen in the hydropic β cells in alloxan diabetes and Duff and Toreson[22] have made the remarkable observation that the deposition of glycogen in the islands can be prevented or reversed by small doses of insulin even in the presence of hyperglycemia They conclude that hypoinsulinemia may be more important than hyperglycemia in producing this change

Goldner and Gomori[45] described marked hydropic change in epithelium of the intralobular ducts of dogs made diabetic with alloxan and examined after sixteen to eighteen days Duff and Starr[46] observed mitotic figures reaching a peak at seventeen hours in the acinar tissue of hooded rats Usually the acini of the pancreas show no change In animals which recover from transient diabetes induced by a single dose of alloxan there is apparently active regeneration of β cells[47] reaching a peak on the fourth day as indicated by colchicine[48] This regenerative activity appears to be increased by the administration of anterior pituitary extract[48,49,50]

Ruangsiri[51] reported observations of the acute effect of alloxan on the living pancreas treated with neutral red and janus green He described clefts of fluid in the β cells in less than thirty minutes after injection of the drug These clefts subsequently enlarged and fluid escaped into the intercellular spaces at the same time that the cytoplasmic granules were disappearing Later fragmentation of nuclei was seen Attempts to confirm these observations in this laboratory have been unsuccessful because of inability to achieve adequate visualization of the β cells *in vivo*

The kidney is perhaps next in importance to the pancreas as a site of lesions in alloxan diabetes The severest damage occurs in the convoluted tubules and appears to be generally proportional to the size of the dose Th epithelium of the tubules may show changes varying from slight swelling and vacuolation to severe necrosis and desqu mati n le ding at times to death of the animal in uremia These findings are apparently limited to abc ... and the renal lesions usually disapp ... sible nature ... tubules may be found if the terminal blood sugar is ...

or higher[42] Intercapillary glomerulosclerosis has not been reported However, Mann and Goddard[43] have found a somewhat similar change, consisting of thickening of the basement membrane deposition of a hyaline material between the capillaries and unlike the human lesion, glomerular adhesions and proliferation of endothelial and epithelial cells (*See* Chapter 12)

Lesions of the liver are apparently unimportant in animals given ordinary doses of alloxan although focal necrosis is described Dogs given large doses may exhibit jaundice and other evidence of hepatic damage In dogs which have been diabetic for some weeks fatty change in the liver may be observed but since fatty livers are also observed in depancreatized dogs this observation is of questionable significance in relation to the direct effects of alloxan Necrosis observed occasionally in dogs cats and rats is more probably due to the drug Herbut *et al*[44] have described severe necrosis leading to portal cirrhosis with deposition of iron resembling hemochromatosis in rabbits given a high cholesterol diet following the injection of alloxan Liver glycogen is said to be normal or even increased in alloxan diabetes[45] but this may vary depending on the severity of the diabetes

In the adrenal glands focal necrosis of both cortex and medulla has been reported The thyroid may be somewhat atrophic Lesions of the eyes include cataracts similar to those of man Necrosis of the basophil cells of the pituitary may follow massive doses of alloxan Arteriosclerosis of severe degree has not been reported in fact alloxan diabetes seems actually to inhibit the development of cholesterol atherosclerosis in the rabbit[46] (*see also* Chapter 9) Alloxan diabetes has caused dwarfing of young rats and abortion and stillbirths have occurred in a large percentage of pregnant diabetic rabbits[47]

It is usually stated that the human islets are resistant to the action of alloxan This opinion as Lukens[7] points out seems to be based chiefly on the work of Brunschwig *et al*[48] who gave alloxan in varying doses to a patient with islet cell carcinoma and to four other patients with advanced malignant disease of other types without producing diabetes or characteristic lesions of the islets at necropsy These investigators however gave the drug in large amounts of saline and therefore slowly In view of the known rapidity with which alloxan is destroyed in the blood stream the doses which they used can hardly be considered comparable to those successfully employed in the experimental animal In two other cases[49,50] definite relief of hypoglycemia has followed the use of alloxan and in one case reported by Conn *et al*[50,51] although an islet cell tumor was found to be resistant the islets in the remainder of the patient's pancreas were damaged and permanent diabetes ensued This patient received a total of 800 mg of alloxan per kg over a period of nine days When the tumor was removed the $\beta$ cells of which it was largely composed were found to be apparently intact while the $\beta$ cells of the islets proper showed varying de-

grees of degeneration from degranulation and hydropic change to loss of cell membranes, pyknosis and fragmentation of nuclei, and complete disappearance of cells. These authors conclude that the sensitivity of the β cells of man is comparable to that of susceptible animals and that alloxan should not be used as a therapeutic agent.

It seems clear that the diabetes which follows injection of alloxan is due essentially to partial pancreatectomy induced by chemical means. However, the exact mechanism of action of the drug on the islets and the cause of its remarkable specificity for the β cells are unknown.[2,7,11] As noted above, the effect of alloxan occurs within five minutes after intravenous injection, as shown by its rapid disappearance from the blood and by the prevention of its action by clamping the pancreatic vessels. Its effect is clearly not due to the triphasic blood sugar curve described above, since prevention of the initial hyperglycemic phase (by insulin, phlorhizin or other means) does not prevent the lesions of the islets or the ensuing

liminary ligation of the pancreatic duct, for reasons which are not clear.[62,63]

As stated by Bruckmann and Wertheimer,[27] "The main difficulty in the elucidation of the mode of action of alloxan lies in our lack of knowledge of the specific biochemical processes of the target tissue, the islet." These authors list three possible mechanisms: (1) selective accumulation in toxic amounts of the drug by the β cells; (2) competition with a structurally similar compound for an enzyme with resultant disorganization of cell metabolism and disintegration of cell structure; (3) specific reaction of alloxan in the islet cell with or without a specific accumulation, as, e.g., by inactivation of an enzyme system concerned with insulin synthesis. An excellent discussion of these theoretical considerations is given by Lazarow[11] (see also Chapter 26), and Houssay[64] presents a detailed discussion of the role of sulfur compounds in carbohydrate metabolism.

Of great interest is the recent production of diabetes with dehydroascorbic acid, a substance which occurs naturally in the body and which is similar in structure and chemical properties to alloxan. This diabetes can also be prevented with glutathione and related compounds.[65,66]

On the supposition that alloxan may act by combining with zinc, Kadota[67] has tried other organic substances reacting with zinc and has claimed successful production of diabetes in rabbits with oxine and dithizone. The histological changes in the islets are said to be similar to those produced by alloxan.

**Pituitary Diabetes.**—In 1927 Johns, O'Mulvenny, Potts and Laughton[8] described a temporary diabetes following the injection of an extract of the anterior lobe of the pituitary in dogs. This report was followed in 1932 by papers from three different groups of investigators on the production of

pituitary diabetes[69, 70, 71] Finally in 1937 Young[7] made the important observation that daily injections in dogs of crude extracts of anterior pituitary over a period of weeks would lead to a permanent diabetic state which persisted after discontinuance of the injections Richardson and Young[72] described lesions of the islets in such dogs

The physiology of pituitary diabetes is complex and has recently been reviewed by Lukens[1] Young[12] and Bennett and Evans[13] to whose papers the reader is referred for further details The thyrotropic lactogenic and gonadotropic factors of the anterior pituitary appear to have no diabetogenic effect and the production of diabetes seems to depend on a combination of the growth and adrenotropic hormones Recently it has been shown that purified growth hormone at least in some animals is a more potent diabetogenic agent than ACTH[13, 74, 75, 76] The mechanism is obscure but seems to involve both suppressed utilization and overproduction of carbohydrate Inhibition of hexokinase may play a part in the process[77]

When a dog is given daily injections of a suitable crude aqueous extract of anterior pituitary hyperglycemia occurs associated with a progressive loss of granulation in the $\beta$ cells a process which requires about seven days to reach its maximum At about this time hydropic change (glycogen[21]) appears The diabetes is now reversible and if injections are stopped recovery may occur and the granules may reappear in the $\beta$ cells If however the injections are continued permanent diabetes (called metahypophyseal diabetes by Houssay[5] and Young[7]) will usually ensue in two to four weeks Thereafter discontinuance of the injections does not affect the diabetes and when the pancreas is examined the islets are found to be small and composed almost entirely of $\alpha$ cells As in the diabetes following subtotal pancreatectomy hydropic change may be seen in the epithelial cells of the small ducts also and in the early stages mitotic figures may be seen in these cells as well as in the $\beta$ cells[78] Hyalinization of the islets has been recorded[79, 17] but is exceedingly rare in contrast to the human disease Intercapillary glomerulosclerosis has been described in the kidneys of a dog maintained in the diabetic state for five years[80]

The ameliorating effect of pituitary extract in alloxan diabetes as observed by Ogilvie[49, 50] and Cavallero[61] has not been elucidated It is perhaps due to a pancreatropic agent (the growth factor?) Many species of animals do not respond with diabetes to the injection of anterior pituitary extract In young rats and rabbits this treatment leads to an increase in the volume of islet tissue and an increase in insulin content of the pancreas a phenomenon which Marks and Young[81] ascribed to a pancreatropic factor in the extract In the toad injection of pituitary extract or implantation of anterior pituitary has no diabetogenic effect when the pancreas is intact, but leads to marked increase in severity of pancreatic diabetes[6]

Purified growth hormone is diabetogenic in the intact adult dog and cat but not in the puppy or kitten In other species its effect is variable[12] These interesting species differences are probably related to similar differ

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Of great interest is the recent production of diabetes with dehydroascorbic acid a substance which occurs naturally in the body and which is similar in structure and chemical properties to alloxan This diabetes *can also be prevented* with *glutathione and* related compounds[65,66]

On the supposition that alloxan may act by combining with zinc Kadota[67] has tried other organic substances reacting with zinc and has claimed successful production of diabetes in rabbits with oxine and dithizone The histological changes *in the islets are said to* be similar to those produced by alloxan

**Pituitary Diabetes** —In 1927 Johns O Mulvenny, Potts and Laughton[68] described a temporary diabetes following the injection of an extract of the anterior lobe of the pituitary in dogs This report *was followed in* 1932 by papers from three different groups of investigators on the production of

pituitary diabetes[69 70 71] Finally in 1937 Young[7] made the important observation that daily injections in dogs of crude extracts of anterior pituitary over a period of weeks would lead to a permanent diabetic state which persisted after discontinuance of the injections Richardson and Young[72] described lesions of the islets in such dogs

The physiology of pituitary diabetes is complex and has recently been reviewed by Lukens[1] Young[1] and Bennett and Evans[13] to whose papers the reader is referred for further details The thyrotropic lactogenic and gonadotropic factors of the anterior pituitary appear to have no diabetogenic effect and the production of diabetes seems to depend on a combination of the growth and adrenotropic hormones Recently it has been shown that purified growth hormone at least in some animals is a more potent diabetogenic agent than ACTH[13 74 75 76] The mechanism is obscure but seems to involve both suppressed utilization and overproduction of carbohydrate Inhibition of hexokinase may play a part in the process[77]

When a dog is given daily injections of a suitable crude aqueous extract of anterior pituitary hyperglycemia occurs associated with a progressive loss of granulation in the $\beta$ cells a process which requires about seven days to reach its maximum At about this time hydropic change (glycogen?) appears The diabetes is now reversible and if injections are stopped recovery may occur and the granules may reappear in the $\beta$ cells If however the injections are continued permanent diabetes (called metahypophyseal diabetes by Houssay[5] and Young[1]) will usually ensue in two to four weeks Thereafter discontinuance of the injections does not affect the diabetes and when the pancreas is examined the islets are found to be small and composed almost entirely of $\alpha$ cells As in the diabetes following subtotal pancreatectomy hydropic change may be seen in the epithelial cells of the small ducts also and in the early stages mitotic figures may be seen in these cells as well as in the $\beta$ cells[78] Hyalinization of the islets has been recorded[79 87] but is exceedingly rare in contrast to the human disease Intercapillary glomerulosclerosis has been described in the kidneys of a dog maintained in the diabetic state for five years[80]

The ameliorating effect of pituitary extract in alloxan diabetes as observed by Ogilvie[49 50] and Cavallero[48] has not been elucidated It is perhaps due to a pancreatropic agent (the growth factor?) Many species of animals do not respond with diabetes to the injection of anterior pituitary extract In young rats and rabbits this treatment leads to an increase in the volume of islet tissue and an increase in insulin content of the pancreas a phenomenon which Marks and Young[81] ascribed to a pancreatropic factor in the extract In the toad injection of pituitary extract or implantation of anterior pituitary has no diabetogenic effect when the pancreas is intact but leads to marked increase in severity of pancreatic diabetes[8]

Purified growth hormone is diabetogenic in the intact adult dog and cat but not in the puppy or kitten In other species its effect is variable[12] These interesting species differences are probably related to similar differ-

ences in the response to partial pancreatectomy which in turn may perhaps be correlated with the habits and environment of the animals [53 54 55]

Lukens and Dohan[56 57] were able to produce diabetes in cats in which one half to three fourths of the pancreas had been previously removed The pathological changes were similar to those in the dog but the phase of hydropic change persisted for about three months before the cells disintegrated and the diabetes became irreversible There is thus a considerable period in case of the cat in which procedures designed to cure the diabetes may be tried During this three months Lukens and Dohan[57] were able to cause disappearance of diabetic symptoms and restoration of the islets to practically a normal state by means of (1) insulin (2) phlorhizin (3) diet and (4) adrenalectomy After the permanent phase of atrophy of the islets had supervened treatment was of no avail

The pathology and pathogenesis of pituitary diabetes have been carefully studied by Ham and Haist [78] These authors noted the resemblance of the lesions to those described by Allen[17] following subtotal pancreatectomy and like him postulated excessive function due to some unknown stimulus as the effective cause of the degranulation hydropic change and final disappearance of the β cells Ham and Haist suggest that pituitary extract may increase the need for insulin by promoting overproduction and under

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are involved in the sequence an early phase, due to increased need for insulin and the late permanent phase due to decreased production of insulin (these would correspond to the idio- and metahypophyseal types) Diabetes could thus be considered in either case as due to increase of the ratio of insulin need to insulin production

Lukens and his co-workers have submitted evidence that hyperglycemia may be the effective stimulus to the β cells They find that the lesions of the β cells can be prevented if the blood sugar is kept at normal levels and that the hydropic change can be reversed by procedures that reduce the

that since both can be used to cure pituitary diabetes ... ions in the early stages it is likely that the level of the blood sugar is a major factor in damaging the islets And yet as Lukens[58] points out in a more recent paper if this be true it is not clear why more cases of human diabetes do not increase in severity nor why the metahypophyseal diabetes of dogs does not get worse Furthermore as noted above, Duff and Toreson[73] have shown that insulin will reverse hydropic change in alloxan dia

... reviewed recently by Hous ... ugar may be seen clinically in

hyperthyroidism and may follow thyroid feeding in animals permanent diabetes has not been produced by this method in intact animals However when the resistance of the pancreas has been reduced by partial pancreatectomy administration of alloxan[34] or anterior pituitary extract the feeding of thyroid will then either intensify a pre-existing diabetes or induce the diabetic state If the pancreatic tissue is reduced in an animal by removal of 6 7 or 7/8 of its mass insufficient in itself to produce diabetes the animal is sensitized to thyroid treatment which may then lead to hyperglycemia glycosuria and ketonuria As treatment is continued lesions of the $\beta$ cells of the islets may appear consisting of loss of granules hydropic change pyknosis and cytolysis In the early stages as in some other forms of experimental diabetes these lesions are reversible and cessation of thyroid feeding leads to recovery of the animal This stage is called by Houssay thyroid diabetes If however the feeding of thyroid is continued over a period of several weeks the lesions may become irreversible and the diabetes may remain permanently even though no more thyroid be given This permanent irreversible phase Houssay terms metathyroid diabetes it is characterized by complete degeneration of $\beta$ cells This phase of the diabetes becomes progressively worse presumably due to the continuing gradual destruction of the $\beta$ cells

The mechanisms involved in the first or extrapancreatic phase of thyroid diabetes are obscure but may be related to the ability of thyroid extract to increase intestinal absorption of glucose and to decrease liver glycogen Later in the metathyroid stage a permanent pancreatic diabetes ensues (as in pituitary diabetes) presumably due to the injurious effect on the $\beta$ cells of the long-continued hyperglycemia or of factors intimately associated with the hyperglycemia

**Administration of Glucose** —The constant association of hyperglycemia with lesions of the $\beta$ cells produced by any means other than alloxan has been noted above Recently Dohan and Lukens[89, 90] have succeeded in producing hydropic change of the $\beta$ cells and permanent diabetes in cats solely by administration of glucose Although this work is as yet unconfirmed its implications are of such importance that it is included here as a fourth method for the production of permanent diabetes These authors using both normal and partially pancreatectomized cats were able by means of repeated intraperitoneal injection of 20 per cent glucose in saline to produce sustained hyperglycemia and hydropic change of the $\beta$ cells They report animals (both normal and partially depancreatized) maintained in hyperglycemia for periods varying from several days to several weeks The development of islet lesions was related to the degree and particularly to the duration of the hyperglycemia One normal animal was kept in a hyperglycemic state for thirty nine days and remained severely diabetic until twenty two days after the last injection when it was sacrificed while in diabetic acidosis

The question of whether the elevated blood sugar stimulates the β cell to the point of functional exhaustion or whether some other mechanism is involved remains to be answered. The authors point out that after the development of hyperglycemia this type of diabetes is quite similar in its course to pituitary diabetes. However in the initial stages there are certain differences as follows. In the first three or four days before lesions of the islands appear crude anterior pituitary extract lowers the R Q inhibits the action of insulin on the blood sugar promotes ketogenesis and thyroid and adrenal hyperplasia occur. There is probably a direct inhibition of the secretion of insulin and the insulin content of the pancreas is reduced. In contrast the administration of glucose initially raises the R Q increases the efficiency of injected insulin is antiketogenic and is not accompanied by thyroid or adrenal hyperplasia. Furthermore glucose stimulates insulin secretion and carbohydrate diet maintains a high insulin content of the pancreas.[90] (*See also* the more recent discussion of hyperglycemia by Lukens.[85])

## METHODS OF PRODUCING TEMPORARY DIABETES

There are of course numerous methods for producing a temporary elevation of the blood sugar (*e g* various drugs and hormones the *piqure* experiment of Claude Bernard). Four of these seem of sufficient theoretical interest to be mentioned briefly.

**Diabetogenic Activity of the Adrenal Cortex** — A possible diabetogenic effect of the adrenal cortex was long suspected because (a) adrenalectomy diminishes the severity of pancreatic diabetes in certain animals (b) conversely administration of adrenal cortical extract to animals having a pancreatic diabetes previously attenuated by adrenalectomy or hypophysectomy increases the severity of symptoms and (c) clinical diabetes is often associated with overactivity of the adrenal cortex as *e g* in Cushing's syndrome and the Achard Thiers syndrome or in association with adrenal cortical tumors.[5, 91]

When purified adrenal cortical hormones and the adrenotropic hormones of the pituitary became available a considerable stimulus was given to attempts at producing experimental diabetes with these substances. The C 11-oxygenated adrenal cortical steroids seem to act by stimulating gluconeogenesis from protein and probably also by inhibiting the utilization of carbohydrate. In an effort to determine the relative importance and possible interaction of these two factors Ingle *et al* [9] studied the effects of insulin with particular reference to nitrogen excretion in intact animals given adrenal cortical steroids and in depancreatized animals. The results of the study were difficult to interpret due to the high resistance to insulin shown by the animals with adrenal steroid diabetes. The only report of lesions of the islets in this type of experiment is as far as we are aware that of Kobernick and More [92] who found striking hydropic change (glycogen)

in the islets of rabbits given cortisone We have been able to confirm this observation in the rabbit (*see* Figs 13 14 and Plate III)[94]

Of great interest is the recent experimental production of temporary diabetes in human volunteers by the intramuscular administration of purified adrenocorticotropic hormone for several days[95,96] Not only hyperglycemia and glycosuria have been produced but also diabetic glucose tolera[illegible]

relationship between uric acid and alloxan the fact that alloxan causes a fall in blood glutathione and the recent experimental work of Griffiths[99,100] who produced hyperglycemia in rabbits by first lowering the blood glutathione by deficient diet and then giving uric acid intraperitoneally (*see below*)

The mechanisms underlying the metabolic changes produced by adrenocorticotropic hormone in man are obscure It seems clear that increased gluconeogenesis from protein does not explain the whole picture there appears to be a general decrease in the ability of the tissues to dispose of glucose normally Conn *et al*[96] suggest the possibility that a heightened intracellular production of uric acid and its intermediaries produced by adrenocorticotropic hormone together with a fall of the levels of glutathione and perhaps other sulfhydryl bearing substances may diminish enzymatic production or release of pancreatic insulin

**Estrogens** —Apparently estrogens may have a mildly diabetogenic effect in depancreatized animals of certain species[101,102] but evidence is conflicting[103,104]

**Uric Acid Diabetes** —In two recent as yet unconfirmed reports Griffiths[99,100] describes the production of hyperglycemia lasting four or five days by the injection of uric acid intraperitoneally into rabbits in which the blood glutathione had previously been lowered by a diet deficient in methionine and cystine The initial blood sugar curve was said to resemble that produced by alloxan These results if confirmed would be of considerable theoretical interest in view of the lowering of blood glutathione which follows the injection of alloxan the prevention of the action of alloxan by glutathione and the effects of adrenotropic hormone on uric acid and glutathione cited above

Collins Williams and Bailey[104] were unable to confirm Griffiths results The suggestion of Griffiths[100] that this may have been due to their use of an anemic breed of rabbits does not seem entirely convincing Grunert and Phillips[105] found that uric acid was not diabetogenic in rats on a diet deficient in methionine and sodium They admit that they were unable to manipulate the diet so as to produce a low glutathione level in both blood and liver (presumably necessary for uric acid to be converted to an alloxan like substance in the liver) but cite unpublished work of their own

in which uric acid was found to be converted quantitatively to allantoin by the livers of sulfhydryl-deficient rats

**Experimental Obesity** —It has been shown that experimental obesity produced by hypothalamic lesions[106] may be associated with diabetes[107] thus producing a striking parallel with the known importance of obesity in man

**Summary** —As noted at the beginning of this chapter it seems clear that all *forms of permanent* irreversible experimental diabetes are associated with and dependent upon lesions of the $\beta$ cells in the islets of Langerhans The various methods for producing diabetes in the experimental animal may be grouped according to whether the damage to the islets is (a) primary or (b) secondary

In the first group belong total and subtotal *pancreatectomy and alloxan* diabetes which is essentially chemical pancreatectomy with respect to the $\beta$ cells In these cases there is a direct and immediate reduction of the total
[illegible]

In the secondary group may be placed the experimental diabetes produced by anterior pituitary extract thyroid feeding and the administra*tion of excessive amounts of glucose In these cases the diabetes is at first* temporary and reversible and if the exciting agent is discontinued during the early stages symptoms disappear and the animal returns to an apparently normal state This may be termed the extrapancreatic phase during which the $\beta$ cells may show the reversible hydropic change (glycogen) due perhaps to the effect of an increased need of the tissues for insulin If however the administration of the exciting agent is continued for a sufficient length of time a permanent pancreatic phase ensues accompanied by lysis and disappearance of the $\beta$ cells

With respect to this secondary group it is noteworthy not only that the same type of lesion in the islets is produced by a variety of agents but also *that there is one factor common to all and apparently essential namely prolonged hyperglycemia* When this is prevented as by starvation or phlor*hizin damage to the islets does not occur* Conversely if the blood sugar is elevated by a high carbohydrate diet or other means the lesions of the $\beta$ cells are accelerated and intensified It seems probable therefore that either the hyperglycemia itself or some *factor constantly associated with it* is responsible for the lesions However the problem is a complex one and hyperglycemia alone may not be the answer[88] The work of Duff and Toreson[23] cited above in which reversal of hydropic change in alloxan dia
[illegible]

Also the important question *of the role of* [illegible] hormone the hyperglycemic-glycogenolytic (*H G*) *factor, is yet to be elu*cidated[108 109] (*see* next chapter *for further discussion*)

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Chapter 26

# ETIOLOGY AND PATHOGENESIS OF DIABETES

This difficult subject is ably discussed by Marble[1] Long[2] Marks[3,4] Soskin[5] Himsworth[6] Lukens[7,8] Wilder[9] and Thorn and Forsham[10] Peters[1] wisely cautions against drawing too hasty generalizations from animal experiments regarding the pathogenesis of the disease in man

However diverse the underlying etiological factors may be it is probable that diabetes mellitus has one immediate cause insulin deficiency This deficiency may be actual through lessened production through neutralization of insulin by hypothetical substances by failure of insulin transport from the islands or it may be relative through increased demand or through insufficient facilities for the formation and storage of glycogen This is succinctly stated by Long[2] as follows It is probably correct to say that the continuance of the disorder of metabolism known as diabetes mellitus rests on a disproportion between the requirements of the organism for insulin and the capacity of the islets of Langerhans to meet this demand This is not to say that diabetes is a disorder of carbohydrate metabolism alone nor does it imply that insulin is the only hormone and the pancreas the only gland involved in the syndrome The major role played by the liver in regulating the blood sugar and the apparent antagonism between the anterior pituitary and adrenal glands on the one hand and the pancreas on the other are emphasized especially by Soskin[5]

## THE PANCREAS

The evidence is overwhelming that whatever the distribution of insulin in the tissues whatever the possible sources of supply the islands of the pancreas are of outstanding importance for insulin production In fact it now seems reasonably clear from the recent work of Wrenshall *et al*[1] that the extent of granulation of the cytoplasm of the $\beta$ cells is closely re*lated to the pancreatic content of insulin suggesting strongly that the* $\beta$ granules actually represent stored insulin If this be true it is natural to look first for lesions of the islands and particularly of the $\beta$ cells in diabetes

At one end of the scale we have the relatively easily understood cases of diabetes developing after extensive destruction of pancreatic tissue either suddenly (and rarely) as in acute pancreatitis or gradually as in hemochromatosis At the other end we have the cases so difficult of explanation in which the various organs are apparently normal although the diabetes is severe However even here insulin deficiency is demon

strated by the abnormal glycogen distribution and by the therapeutic effect of insulin.

Further evidence for the importance of the pancreas in most cases of human diabetes is the fact that the diabetic pancreas usually contains far less insulin than normal,[12,13] also that diabetics usually excrete less insulin in their urine,[14] although this could be due to an increased rate of destruction. Also it should be stated again with emphasis that in all forms of permanent experimental diabetes *without exception* lesions of the islands of Langerhans are demonstrable (*see* Chapter 25).

Anatomical causes of insulin deficiency may be listed as follows:

- I. Destruction of pancreatic tissue (both insular and acinar)
  - 1. Pancreatitis
    - a) Acute
    - b) Chronic (with or without calculi)
  - 2. Malignant disease
    - a) Primary
    - b) Metastatic
  - 3. Hemochromatosis
  - 4. Toxic injury
  - 5. Surgical removal
- II. Selective destruction of insular tissue
  - 1. Hyaline infiltration
    - a) Amyloid infiltration
  - 2. Fibrosis
  - 3. Toxic injury with lymphocytic infiltration
  - 4. Hydropic change (glycogen infiltration)
  - 5. Reduction in $\beta$ cells due to unknown causes
- III. Inadequate insular tissue (congenital deficiency)
- IV. Inadequate blood supply
  - 1. Arteriosclerosis

Most of these lesions have been discussed in Chapter 3 and need not be considered in detail here.

The effect of total pancreatectomy in man is of great theoretical interest. Several well attested cases which survived for a reasonable period are now available and it seems established without question that total pancreatectomy in man produces a diabetes requiring about 40 units of insulin daily for control.[15,16,17] This finding is contrary to expectations since it was formerly thought that total diabetes (*i.e.* that associated with complete loss of insular tissue) would require large amounts of insulin (about 200 units per day on the basis of animal experiments). The fact that it does not invites speculation as to the importance in the usually encountered severe case of diabetes of (*a*) the external secretion of the pancreas, (*b*) contra-insulin factors or hormones, (*c*) peripheral destruction or inactivation of insulin, (*d*) greatly increased metabolic processes requiring large amounts of insulin and (*e*) the possible effect of the still hypothetical hyperglycemic-glycogenolytic hormone of the $\alpha$ cells. With regard to (*a*) above, the obvious factor of malnutrition in the animal or man totally deprived of his pancreas is not to be neglected. It should [illegible] ized also

that insulin requirement is not the only nor necessarily the most valid criterion of the severity of diabetes (*See* further discussion on page 296.)

The mechanism by which selective destruction of insular tissue takes place is obscure. Hydropic change (glycogen infiltration[18]) of the β cells is the characteristic lesion of experimental diabetes no matter how produced, and seems to be associated with, if not caused by, hyperglycemia, especially in view of the fact that the administration of excessive amounts of glucose alone will produce the lesion (however, the effect of hyperglycemia cannot be reduced to a simple formula (*see below*). The emphasis of Mirsky[24] and others on the rarity of this lesion in human diabetes would seem to have little relevance, since obviously experimental conditions do not obtain, and the stimulus, whatever it may be, probably acts over a period of *years rather than days or weeks*. Furthermore, it is rare that a fresh, newly discovered case of diabetes (*i.e.*, the type in which one would expect hydropic change) comes to autopsy. Also, if it be true, as the work of Duff and Toreson[19] suggests, that insulin can reverse this change even in the presence of hyperglycemia, then one would expect the lesion to be rare in treated patients.

Speculation concerning possible causes for the selective destruction of β cells in the islands (*see* Chapter 3 for evidence that β cells are reduced in number in many cases of diabetes) was greatly stimulated by the finding that alloxan in proper doses causes such destruction (*see* Chapter 25). The possible role of alloxan, or a closely related substance, in the etiology of diabetes has been elaborated particularly by Lazarow.[20,21] This author [illegible]

observations he suggests that the specialization of the β cells for insulin synthesis may result in a localized depletion of glutathione in these cells. He points out that insulin is 12 per cent cystine and depends on the S-S group for its activity, and that the cystine can be formed by oxidation of cysteine, which is also a constituent of glutathione. Because of this situation, there may be competition for the cysteine reaching the β cells, since it is used for synthesis of both insulin and glutathione. As a consequence of this competition, Lazarow suggests there may be less cystine available in the β cells for *glutathione synthesis than is the case with other cells*. It has been further calculated that if the cysteine contained in glutathione

has summed up these hypotheses in a diagram [illegible]

Houssay[22] has recently published a detailed and well-documented survey of the relation of sulfur compounds to carbohydrate *metabolism*, with particular reference to glutathione, the SH-containing enzymes, and the effects of alloxan.

Heightened interest in the possible rôle of alloxan (or a closely related compound) was also provided by the experimental work of Griffiths [23,24] who has claimed to produce diabetes in rabbits with uric acid after preliminary depletion of the blood glutathione level by means of a diet deficient in cystine and methionine but containing large amounts of ascorbic acid (*See also* Chapter 25)

Conn [25,26,27] on the basis of his spectacular work in producing a temporary

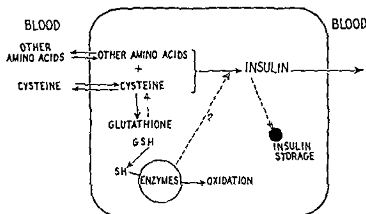



FIG 111 — Metabolism of the $\beta$ cell according to Lazarow (Reproduced by courtesy of Dr Arnold Lazarow from *Physiological Reviews* 29 48 1949)
Amino-acids including cysteine enter the $\beta$ cell from the blood There cysteine is

are inactivated by alloxan the cells may not only lose their capacity to synthesize insulin but the cells themselves may die because they are unable to carry on basic metabolic functions

According to some investigators [28,29] no deviation from the normal has been demonstrated in the uric acid and glutathione levels in the blood of diabetic patients However, Houssay [27] cites several European writers who found a decrease

Leech and Marble [30] have studied the total hydrolyzable cystine in the blood serum of 52 diabetics and 25 normal patients The diabetics showed a trend toward a decreased total cystine in the serum as a whole and in the

that insulin requirement is not the only nor necessarily the most valid criterion of the severity of diabetes (*See* further discussion on page 296.)

The mechanism by which selective destruction of insular tissue takes place is obscure. *Hydropic change (glycogen infiltration*[19]*) of the β cells* is the characteristic lesion of experimental diabetes no matter how produced and seems to be associated with, if not caused by, hyperglycemia, especially in view of the fact that the administration of excessive amounts of glucose alone will produce the lesion (however, the effect of hyperglycemia cannot be reduced to a simple formula (*see below*). The emphasis of Mirsky[34] and others on the rarity of this lesion in human diabetes would seem to have little relevance since obviously experimental conditions do not obtain and the stimulus, whatever it may be, probably acts over a period of years rather than days or weeks. Furthermore, it is rare that a fresh newly discovered case of diabetes (*i.e.*, the type in which one would expect *hydropic change*) *comes to autopsy*. *Also, if it be true, as the work of Duff* and Toreson[9] suggests, that insulin can reverse this change even in the presence of hyperglycemia, then one would expect the lesion to be rare in treated patients.

Speculation concerning possible causes for the selective destruction of β cells in the islands (*see* Chapter 3 for evidence that β cells are reduced in number in many cases of diabetes) was greatly stimulated by the finding that alloxan in proper doses causes such destruction (*see* Chapter 25). The possible role of alloxan, or a closely related substance, in the etiology of diabetes has been elaborated particularly by Lazarow.[70,71] This author was responsible for demonstrating that a large dose of glutathione administered just before a diabetogenic dose of alloxan would protect the experimental animal from developing diabetes. As a result of this and similar observations he suggests that the specialization of the β cells for insulin synthesis may result in a localized depletion of glutathione in these cells. He points out that insulin is 12 per cent cystine and depends on the S-S group for its activity, and that the cystine can be formed by oxidation of cysteine, which is also a constituent of glutathione. Because of this situation there may be competition for the cysteine reaching the β cells, since it is used for synthesis of both insulin and glutathione. As a consequence of this competition, Lazarow suggests, there may be less cystine available in the β cells for glutathione synthesis than is the case with other cells. It has been further calculated that if the cysteine contained in glutathione could be made available for insulin synthesis, then the synthesis of physiological amounts of insulin could deplete the β cells of glutathione and thereby render the enzymes of these cells more susceptible to alloxan. Lazarow ha[illegible] Figure 111 [illegible] h [illegible] of [illegible] particular reference to glutathione, the SH-containing enzymes [illegible] e effects of alloxan.

higher insulin requirement than pancreatectomized animals were able to live longer without insulin and did not develop ketosis and coma As a result of their observations these authors put forth the hypothesis that there is secreted by the pancreas a second endocrine factor which acts to increase the blood sugar but to prevent ketosis in the insulin-deficient animal More recently the H G factor has been studied by Sutherland and co workers[36 37 38] and by Pincus[39 40] Sutherland[41] has recently reviewed the subject concluding that the factor is a protein and in all probability a hormone which raises the blood sugar by promoting glycogenolysis in the liver Sutherland and de Duve[37] were able to obtain the substance not only from commercial insulin preparations but also from the pancreatic tissue of several species and from the upper three-fourths of the gastric mucosa of the dog Since they found a normal or increased amount in the sclerosed pancreas after ligation of ducts (which destroys the acinar tissue but allows the islets to survive) and normal amounts in the pancreas of alloxan-diabetic animals they suggested that it was entirely possible that the substance was produced by the $\alpha$ cells They noted however that the presence of an apparently identical factor in the upper part of the gastric mucosa of the dog raised the question of specificity unless it could be shown that the [illegible]

by this method[42] hence there must be some histochemical difference between the two cell types

Gaede *et al*[43] have described a single dog to which some weeks after ligating the pancreatic ducts they gave alloxan thus producing islets consisting almost solely of $\alpha$ cells embedded essentially in fibrous tissue From this sclerotic pancreas they obtained an extract which raised the blood sugar of a normal rabbit from about 90 to 119 mg per cent They explained the feeble rise in blood sugar by the small size of the islets and concluded that the $\alpha$ cells were indeed the sources of the hyperglycemic factor Such an isolated observation though interesting is of course not conclusive especially since *no control observations on extracts of other tissues are offered* It is perhaps conceivable that some nonspecific substance in the extract having an epinephrine-like effect accounted for the slight rise in blood sugar observed However this seems to be the nearest approach to a pure extract of $\alpha$ cells and seems to fit in with the work of others Lerner[44] has set forth various hypotheses in relation to the possible importance of the H G factor and the total mass of $\alpha$ cells in normal carbohydrate metabolism and in relation to insulin resistance These speculations are stimulating and thought provoking but are as yet unsupported by sufficient evidence to make them acceptable

Pincus[40] apparently with a similar hypothesis in mind gave insulin preparations with the insulin inactivated by heat and alkali to diabetic and normal persons and noted the most marked rise in blood sugar among

albumin and globulin fractions of the serum protein. At all ranges of serum protein concentration the diabetics showed a lower cystine protein ratio than the normals. These authors also found using sodium sulfite fractionation that 46 per cent of 40 fasting diabetics showed an albumin globulin ratio under 1.5 while some of 19 normals had ratios as low. Their diabetic patients showed further a striking variability in serum cystine and serum protein concentrations and in the cystine-protein ratio in contrast to a remarkable constancy in the normal individuals studied. The authors suggest that this variability may be related to the activity of insulin and to the state of control of the diabetes at the time the blood sample was withdrawn. Experimental confirmation of these data was lent by the finding of a low total cystine and low cystine protein ratio in animals with diabetes produced either by alloxan or pancreatectomy and by the tendency of injected insulin to raise the total cystine and the cystine-protein ratios toward their original levels.

To date, of course, all assumptions concerning the intracellular metabolism of the $\beta$ cell are highly speculative. It is possible, too, that all such hypotheses may have to be modified in the light of Toreson's[4] demonstration that many if not all cases of so-called hydropic degeneration of the islands are due to actual intracellular deposition of glycogen. It is conceivable, as indicated elsewhere, that the presence of this glycogen is simply a nonspecific manifestation of storage as occurs in many other cells of the body. According to this point of view it would perhaps be no more surprising to find glycogen in the insular cells than to find it, say, in the iris of the eye (*see* Chapter 13). Yet experimental evidence suggests strongly that in the $\beta$ cells such glycogen deposits are the earliest manifestations of injury.

Toreson[18] makes the interesting suggestion that the glycogen in the islands and in the ducts may be an indication of regenerative activity. As noted elsewhere, Duff and Toreson[19] have made the important observation that insulin in small doses may reverse this change even in the presence of hyperglycemia. They conclude that hypoinsulinemia is a much more important factor in producing the change than is hyperglycemia.

In view of the newer work on the hyperglycemic-glycogenolytic (H G) factor of the pancreas and the suggestion that this hypothetical hormone may be a product of the $\alpha$ cells, it now seems necessary to go beyond the $\beta$ cells in considering the role played by the pancreas in diabetes. In 1923 Murlin and co-workers[31] noted the presence in extracts of pancreas of a substance which would raise the blood sugar and which they named "glucagon."[3] Such a substance was encountered by various investigators as a contaminant of various American and Canadian insulin preparations

... H G

further substantiation of this evidence occasional mitotic figures can be found in the cells of the younger pigment free islands.

The pigment is not restricted to the island cells but affects the acinar tissues as well. The same evidence of regeneration is offered by the acinar cells as by those of the islands.

The well-established evidence of destruction and regeneration of parenchymal cells in the liver offers a striking parallel to the changes in the pancreas in hemochromatosis. Just as in the liver the parenchymal cells show every stage from newly formed pigment free cells through those containing hemofuscin and those containing hemosiderin to necrotic cells, the same steps can be traced in the acinar and island cells of the pancreas.[53]

The pancreas in diabetes is not simply the scarred field of an old battleground but is the actual field of conflict. It does not submit without a struggle to injury but endeavors to regenerate. This leads to the hope that in young people with their more resilient tissues proper treatment may remove the cause of injury and favor more or less complete restoration of the damaged islands.

Arteriosclerosis as an etiologic factor in diabetes is not given much weight in most discussions of the subject. However Moschcowitz[54] holds that hyperplastic and hyaline arterial lesions are more frequent in older diabetics, particularly hypertensive ones, than in nondiabetics of comparable age, and that lesions of the islands (fibrosis and hyalinization) should be regarded as a capillary sclerosis extending from the arteriolar lesions.

## EXTRAPANCREATIC FACTORS

As noted at the outset, an insulin deficiency may be due to (a) actual decrease in output of the pancreas due to a reduction in the quantity of islet tissue, or (b) excessive demand for, or destruction of, insulin so that even though the pancreas is producing a normal or even supernormal amount of insulin the supply is inadequate to meet the demand. The latter condition seems to occur in the early stages of some forms of experimental diabetes, although as previously emphasized, pancreatic lesions can be demonstrated later and must be regarded as responsible for the permanence of the diabetic state. It should be remembered, therefore, that many of the so-called "extrapancreatic" factors listed below exert their effects sooner or later, directly or indirectly, upon the pancreas. In fact, pancreatic damage is probably essential if a permanent diabetes is to follow.

**The Pituitary.** — A possible role of the pituitary in human diabetes has been considered since the classical work of Houssay in showing the ameliorating effect of hypophysectomy on experimental diabetes. The experimental production of diabetes in animals or man, whether by means of crude extracts,[51] purified adrenocorticotropic hormone [illegible] 27 or purified growth hormone[51,56] seems to lead to [illegible] early [illegible] (idiohypophyseal diabetes of Young) in w[illegible] β cells can

patients with labile diabetes McQuarrie *et al*[44] report an interesting condition of familial hypoglycemosis in which $\alpha$ cells are said to be completely *or almost completely absent from the pancreas*

If such an $\alpha$ cell hormone exists it may of course help to explain many previously puzzling observations such as the smaller insulin requirements of dogs with total pancreatectomy as compared with those having a partial pancreatectomy[45] and the similarly reduced insulin requirements of totally pancreatectomized man as compared with natural diabetes[16,17]

However the validity of many of the experiments on which such conclusions are based has been questioned recently by Mirsky *et al*[4a] They hold that pancreatectomy in the alloxan-diabetic dog actually produces an aggravation of the diabetic state since the hyperglycemia glycosuria and ketonemia observed during a period of fasting and exogenous insulin deprivation are greater after than before pancreatectomy in such an animal

In any case the pathology of the pancreas in diabetes cannot be considered as static As seen at any one time it represents a balance struck *between the factors tending to produce degenerative changes of the islands* and the regenerative powers of the pancreas That these latter are by no means inconsiderable has been shown by the experimental work of Bensley[46] and his pupils and the observations of Boyd[47] and is evidenced also by the not infrequent occurrence of mitotic figures in islands when the epithelium has been injured

Considerable regenerative activity may be seen in various types of experimental *diabetes (see Chapter 2o) Haist[48] has described the remarkable* fluctuations which can be produced in the total volume of the islands in the rat by means of various stimuli (*see* Chapter 3) Apparently repeated small doses of insulin may lead to a disuse atrophy of the islets[49] It is conceivable of course that the fundamental defect in the diabetic is an inability to regenerate $\beta$ cells at an adequate rate Species differences in animals are well recognized for instance the immunity of the rat to pituitary *diabetes is supposedly due to its ability to regenerate $\beta$ cells*[4,10,4]

New formation of islands from intralobular duct epithelium has been described by Burkhardt[52] in 2 cases of carcinoma of the ampulla involving the head of the pancreas giving a picture similar to that of duct ligation Most islands were atypical and showed degenerative changes *Both cases* showed clinical diabetes *In both cases the carcinoma probably antedated* the diabetes

The important *role played by hemochromatosis in elucidating* the process one of us has already stressed We find variation in involvement of the islands (in hemochromatosis) ranging from the remains of islands represented by clusters of pigment loaded endothelial leuko-

g

n

formed to take the place of those destroyed by the p[illegible]

Chapter 16) Here it becomes obvious that "pituitary diabetes" and "adrenal diabetes" must overlap since it is uncertain how much of the effect of crude pituitary extracts used in the past may have been attributable to the adrenotropic and how much to the growth factor. Presumably the diabetes produced by ACTH and the associated disturbance of uric acid metabolism is due to overproduction of steroid hormones by the adrenal cortex although this is not established[25,26] The recent observation of hydropic change (glycogen) in rabbits injected with cortisone by Kobernick and More[66] is an important lead (cf Chapters 16 and 25 and Figs 13 and 14)

Because of the associated upset in purine metabolism produced by ACTH and manifested by increase in urinary excretion of uric acid and because of the alleviation of this type of experimental diabetes by glutathione Conn's[26] has hypothesized that a purine metabolite exerting an alloxan like effect is responsible for reducing the intracellular availability of free sulfhydryl groups which are necessary for the normal activity of many enzyme systems that the combination of decreased intracellular concentrations of sulfhydryl groups and increased intracellular concentrations of purine metabolites impairs functional activity of the insulin producing cells of the pancreas (the product of which is rich in cystine) and also interferes with peripheral glucose utilization by inhibition of those enzyme sys-

[illegible]

tered in the adrenals in human diabetes whether functional or anatomical may be entirely nonspecific and secondary to a state of stress in the sense of Selye[67] In any given case therefore it may be impossible to say whether the adrenals have any primary or etiological significance The effect of removal of tumors however is convincing

Recent evidence suggesting that adrenal cortical function may actually be diminished in diabetes is given in Chapter 16 page 202

**The Thyroid** Houssay[68] has shown that a temporary or permanent diabetes can be produced by administration of thyroid extract in animals with already damaged pancreases (Chapter 25) Clinically it is recognized that hyperthyroidism developing in a diabetic may cause exacerbation of the diabetes and myxedema may lead to amelioration Balfour and Sprague[64] give illustrative examples Ricketts[69] suggests that hyperthyroidism appearing in a patient predisposed by heredity or other factors to diabetes may precipitate the disease in persistent form Increased activity of the thyroid would appear to be an excellent example of an extrapancreatic factor producing an excessive demand for insulin since presumably the effects of the thyroid are exerted solely through its influence in speeding up the rate of metabolism

**Neurogenic Diabetes** —The occurrence of emotional glycosuria and the sugar puncture of Claude Bernard point to a close relationship between

usually be found followed by a later permanent metahypophyseal phase in which the $\beta$ cells are destroyed[56] Whether the primary injury to the $\beta$ cells is due to functional overstrain because of an increased demand for insulin to injury by an alloxan like substance appearing as a result of a simultaneous aberration of uric acid metabolism to glycogen deposition or to other unknown factors is not clear at the present time Anderson and Long[57] have shown that growth hormone apparently inhibits the production of insulin by the pancreas It is also possible that the anterior pituitary produces a specific inhibitor of hexokinase thus causing fundamental interference with carbohydrate metabolism[58 59 60] The hypothesis of Lerner[42] that the growth hormone may be alphacytotropic is mentioned above (p 295) Clinical evidence that the pituitary may play a rôle in some cases of human diabetes is provided by the observations of White[6] on increased stature among diabetic children (although Rosenbusch[8] could not confirm this finding in Switzerland) and by the well known increased incidence of diabetes in acromegaly

Bennett and Evans[55] sum up the experimental evidence for involvement of the pituitary in diabetes as follows The four observations which offer the strongest support for the concept that a balance of hormones is involved [i e in carbohydrate metabolism] and that the balance is upset in the metabolic disturbance of diabetes mellitus are first the marked hypersensitivity to insulin that develops in hypophysectomized animals and that cannot be accounted for by secondary hypofunction of the other [illegible] to [illegible] ng [illegible]

physectomy of depancreatized animals and the ability of such doubly operated animals to survive for long periods of time without insulin and fourth the production of temporary or permanent diabetes mellitus in normal animals with crude hypophyseal extracts and reinvocation of severe diabetes mellitus in hypophysectomized pancreatectomized animal by such extracts

[illegible] discussion of the pituitary may be found in Chapters 15 and 2[illegible]

[illegible]

createctomized cats[63] Also an ameliorating effect on human [illegible] been observed with the onset of Addison's disease[64] Additional evidence [illegible] by the well known association of diabetes with tumors of both [illegible]

[illegible]

diabetes has been produced in rats [illegible] humans by Forsham et al and by Conn using ACTH (References to

diabetics than in the general population.[57] Taub *et al.*[58] have claimed to differentiate two groups of diabetics on the basis of liver function tests: the "juvenile" or insulin deficiency type and the "adult" or hepatic dysfunction type, which would presumably correspond to Himsworth's[6] insulin-sensitive and insulin-insensitive types (*see below*). It is not by any means clear that the liver is the major factor in the second group.

It is unlikely that the liver *per se* is of great importance as an etiological factor in the disease. As Himsworth[6] points out, it is usually necessary to destroy practically the entire liver before significant changes in carbohydrate metabolism are produced. Likewise, it is not clear that the lipotropic factors (lecithin, choline, lipocaic) are concerned in the etiology of diabetes.[1]

**Trauma and Stress.** Direct trauma in the sense of injury to the pancreas sufficient to destroy the greater part of the organ is rarely a cause of diabetes.

One of the few evidences for traumatic origin of diabetes is offered by Wells' case,[8] that of a teamster aged thirty-two years who was run over by his wagon. Four and a half months after injury he was brought to the hospital stuporous, seriously ill, and emaciated. It was learned that since the accident he had developed polydipsia and pruritus. Neither blood nor urine was tested for sugar as he was moribund. At autopsy the pancreas weighed 122 grams, was irregular, and felt as though filled with small stones. The duct was not completely obstructed. No concretions were present outside the pancreas.

On microscopic examination fibrosis was extreme, with only traces of acini and a few shrunken, partly fibrosed islands. Throughout, masses of calcified material were surrounded by a dense fibrosis. Most of the calcium appeared to be deposited as a result of necrosis of parenchyma. Over 95 per cent of the pancreatic tissue had been replaced in this way. Hence there was adequate anatomical basis for the diabetes.

Although direct trauma to the pancreas is hardly ever a convincing cause of diabetes, there has been a tendency recently to broaden the concept of trauma to include injury to other parts of the body, severe illnesses, and even psychic stress. Selye[67] has suggested that diabetes may be one of the "diseases of adaptation," and this concept has been enlarged upon by others.[68] It has certainly been amply demonstrated that "emotional glycosuria" occurs,[69] and it may well be that this phenomenon is part of an "alarm reaction" or "adaptation syndrome" mediated perhaps through either the adrenal medulla[70] or cortex[67] or both. McArthur *et al.*[67] have shown that the rate of urinary excretion of corticosteroids during diabetic acidosis may be 2 to 5 times as rapid as after recovery.

However, as noted above, diabetes seems to decrease during war time in spite of the associated stress.

**Obesity and Diet.** The importance of obesity, usually an evidence of chronic overeating, as a predisposing factor in the production of diabetes

the central nervous system and the pancreas. Intermediate action of the adrenals and pituitary in the case of emotional glycosuria is quite possible, i.e., a stress phenomenon in the sense of Cannon[70] and Selye.[67] The World Wars, in spite of tremendous emotional strain on millions of persons, did not provide any noticeable increase in the number of diabetic cases; in fact, a decrease has been reported, presumably due chiefly to the prevalent undernutrition.

*Lesions in the vicinity of the hypothalamus have been reported in a number of cases of diabetes.*

*An ependymal cyst of the third ventricle was found in a diabetic by Byrom and Russell.*[71] Worthy of note is the observation of Gissel[72] that severe head injuries almost always *produce a transient hyperglycemia.* Davidson and Allan[73] found higher blood sugar values after injection of glucose in 12 patients with concussion and in 18 with skull fracture. However, these observations are not confirmed by other neurosurgeons (*cf.* Joslin[74]). At any rate, glycosuria following trauma may well be a nonspecific stress phenomenon (*see below*).

Further evidence of the association of changes in the central nervous system and glycosuria has been brought out by Vonderahe.[75] Three cases presented hemorrhage affecting the tuber cinereum or hypothalamus with *subsequent glycosuria; one case of diabetes of some duration showed a cyst* of the pulvinar of the thalamus; and one case of capillary hemorrhage in *the hypothalamus was accompanied by terminal hyperglycemia.* One case of diabetes recovered following removal of a meningioma impinging on the fourth ventricle.[76]

Blackford and Venable[77] report 2 cases of paresis with diabetes in which they believe the diabetes was secondary to the syphilitic process.

As far as we are aware, the claims of Vonderahe[75] and of Morgan *et al.*[78] that a reduction in ganglion cells can be demonstrated in the paraventricular nuclei in diabetes have not been confirmed.

Whether hypothalamic lesions may act by way of the anterior pituitary or otherwise is not known. *In recent years there has been a tendency to*

[74]

ney to emphasize the

point that the release

*of sugar from the liver* is responsible for the glucose tolerance curve, both normal and diabetic. Forsham and Thorn[79] have suggested that determination of serum inorganic phosphorus simultaneously with a glucose tolerance test may give a clue to liver involvement in diabetes. *Levy et al.*[80] and Zimmerman *et al.*[81] have found evidence of *mild* impairment of function and have also found fatty change *and cirrhosis in a few biopsies* (*see* Chapter 6). But Brown,[8] applying 8 standard liver function tests in 25 diabetics, found no evidence of impairment (choice of tests and interpretation of them are obvious bases for disagreement). Also, there seems to be no evidence that Laennec's cirrhosis of the liver is more common in

whose disease had been discovered during the normal period of growth (growth-onset type) while relatively high values were found in subjects who had reached full stature prior to discovery of the diabetes (maturity-onset type) The authors speculate as to the relative importance of the growth hormone in the two types

The effect of prior diet has been studied extensively by Himsworth[6] who believes that the incidence of diabetes can be related to the amount of fat in the diet although the mechanism by which a high fat diet might bring on diabetes is obscure just as the predisposing effect of obesity *per se* is obscure The idea that a high carbohydrate diet may have etiological significance seems less popular than in previous years in spite of the demonstration that massive amounts of glucose will produce diabetes in animals (*See next section below* )

In this connection the work of Houssay and Martinez[90] is of interest They showed that a diet high in certain types of fat (lard and ox fat) would greatly increase the mortality of rats from alloxan diabetes The effect of diet on the actual volume of islet tissue is noted elsewhere (p 22) The occurrence of diabetes in experimental obesity associated with hypothalamic lesions is mentioned in Chapter 25 (p 286)

**Hyperglycemia** — An excellent discussion of the possible pathogenetic role played by hyperglycemia in both experimental and human diabetes is given by Lukens[85] He concludes that it is impossible to state flatly that hyperglycemia is or is not harmful since any injurious effect may depend on co-existent circumstances For instance under certain experimental conditions a diabetes in the early reversible phase may be cured by either insulin or phlorhizin both of which seem to lower the blood sugar by different mechanisms Also diabetes may be produced by excessive administration of glucose to cats On the other hand if hyperglycemia *per se* is harmful it is not clear why the metahypophyseal diabetes of dogs does not show a rapidly increasing severity nor why mild diabetes in man does not usually become more severe nor why the diabetes associated with pheochromocytoma (Chapter 16) is so readily reversible The work of Duff and Toreson[19] indicating that insulin treatment may reverse hydropic change (glycogen infiltration) of the islets even in the presence of continuing hyperglycemia suggests that some other factor *e g* hypoinsulinemia may be more important On the whole it would seem that an agnostic rather than a dogmatic point of view on this subject is desirable

**Infection** While the temporary unfavorable effect of infectious diseases in diabetes is established beyond all equivocation as a direct cause of the disease it is of little importance Histological resemblances between pancreas and parotid have led to special interest in mumps[91 92] but this disease can be but rarely blamed for diabetes

Severe toxic injury to the islands in the course of various infections may produce either transient glycosuria or more rarely true diabetes Root and Warren[93] have called attention to the actual necrosis of insular epithelium

has been well emphasized by Joslin[74] and others. Obesity may be regarded as indubitable evidence of abnormal food intake and hence of abnormal strain on the islands of Langerhans.

Table 47 illustrates the frequency of obesity.

TABLE 47. VARIATION FROM NORMAL OF MAXIMUM WEIGHTS AT OR PRIOR TO ONSET OF 1000 CASES OF TRUE DIABETES CALCULATED FOR HEIGHT, AGE AND SEX (JOSLIN[7])

| Age years | Number of cases | Percentage in normal average zone (+5 to −5 per cent) | Percentage of each decade | |
|---|---|---|---|---|
| | | | Below standard weight | Above standard weight |
| 0 to 10 | 43 | 37 | 44 | 19 |
| 11 to 20 | 84 | 39 | 29 | 32 |
| 21 to 30 | 112 | 19 | 10 | 71 |
| 31 to 40 | 172 | 6 | 5 | 89 |
| 41 to 50 | 244 | 12 | 3 | 85 |
| 51 to 60 | 252 | 12 | 1 | 87 |
| 61 to 70 | 9 | 10 | 6 | 84 |
| 71 to 80 | 14 | 11 | | 79 |

A striking fact is the usual reversibility of diabetes in the middle-aged obese subject, i.e., the glycosuria may be expected to disappear if weight loss is adequate. In fact, consideration of certain data leads to the startling conclusion that "almost half of the diabetics extant could be removed from this category simply by cure of their obesity."[88]

*The frequent association of diabetes with obesity, especially in the mid* ... rate classification of this group. Hims... and ... insulin insensitive types, the ... h an unstable, severe diabetes respond-ing readily to insulin, the latter usually middle-aged and obese and tolerating large doses of insulin without symptoms. The former he considers as possibly due to insulin lack, the latter to insensitivity to insulin. *There is* an obvious parallel between the latter group and animals in the "idiohypophyseal" stage of pituitary diabetes. Of particular interest in this connection are the recent observations of Bornstein and Lawrence[89] who carried out insulin assays by a new method on the blood of two groups of patients, one the young, severe type with ketosis and requiring insulin to live, the other the middle-aged obese type without ketosis and controllable ... while all of ... plications of

Also important is the recent work of Wrenshall ... studied the extractable insulin, as measured by a mouse convulsion or a mouse blood sugar method, in a series of pancreases from diabetic and nondiabetic individuals. The lowest values relative to controls were found in diabetics

Harris[99] holds the problem to be more complex than hitherto assumed

7 per cent of the siblings of diabetics whose age at onset of the disease is less than 30 may be expected to develop diabetes by age 40 while only about 1 3 per cent of the siblings of those who develop the disease after the age of 30 may be expected to have diabetes by age 40 He suggests that many of the late onset type may be heterozygous for a gene which in homozygous form may give rise to the severe early onset type of case

TABLE 48 INCIDENCE OF DIABETES IN THE FAMILIES OF DIABETICS PERCENTAGE OF CASES REPORTING HEREDITARY AND OR FAMILIAL TYPES OF FAMILY HISTORY RECENT CLINICAL EXPERIENCES (JOSLIN[71])

| | *No of cases* | *Per cent with family history of diabetes* |
|---|---|---|
| Joslin total children 1946 | 2191 | 35 0 |
| Joslin 1946 Children of 20 or more years duration | 249 | 55 0 |
| Joslin 1941 Hospital cases | 1619 | 41 0 |
| Joslin 1920 1928 | 4831 | 25 6 |
| Barach Pittsburgh 1938 | 1030 | 41 5 |
| John Cleveland 1938 1939 | 630 | 35 7* |
| Lawrence London to January 1933 | 5462 | 32 3 |
| U S Veterans Bureau to August 1931 | 704 | 7 0† |

* Includes 7 cases with combined hereditary and familial history
† Includes 35 cases with combined hereditary and familial history

Barker *et al* [100] describe a family in which 3 boys all developed diabetes at an early age while 3 girls were spared They suggest inheritance of the disease as a sex linked recessive with heterozygous parents

## CASES WITH NO SIGNIFICANT PATHOLOGY

There remains to be explained the group of cases without definite anatomical change in the pancreas or other organs Even the granules in the island cells may appear normal In our series of 811 diabetic autopsies the pancreas was normal as far as could be determined in 271 or almost exactly a third of all cases (In most of these the newer staining methods were not used ) No lesions in other organs were sufficient to explain the existence of diabetes although the pituitary was examined in only a small proportion However no evidence of clinical disturbance other than the diabetic process was encountered

These cases in which changes in the pancreas are absent or too trifling to merit consideration have always puzzled and irritated the pathologist It

that may occur in such cases and also to evidences of *regeneration* Of [illegible]

[illegible] *prob-*
[illegible] present in all cells of the body and catalyzes a key reaction by which glucose is phosphorylated The anterior pituitary is *thought to* produce a specific inhibitor of hexokinase [8,59,60] also one of the actions of the oxygenated steroids of the adrenal cortex appears to be the enhancement and prolongation of the action of this pituitary hexokinase inhibitor [93] It has been further held that a decrease in hexokinase activity could be demonstrated in the muscle and liver of the alloxanized rat [60]

The possible rôle played by these reactions in the pathogenesis of human diabetes must await further work especially since others[94] have had difficulty in completely confirming the original observations of Cori *et al* [59]

**Insulinase** —Recently Mirsky and co-workers [95] in a series of papers have described an enzyme system (insulinase) which inactivates insulin *Apparently fasting diminishes the liver insulinase and feeding reconstitutes it* The possible place of this enzyme in the physiology of human diabetes is not yet clear

**Phosphatases** —Since these enzymes are of undoubted importance in carbohydrate metabolism especially in the dephosphorylation of glucose it is natural to consider whether they are involved in the pathogenesis of diabetes Drabkin[96] considers that they are and holds that a relationship can be demonstrated between the level of the blood sugar and the phosphatase activities of the kidney blood serum and liver Also the apparent increase in liver phosphatases in alloxan diabetes and their restoration to normal suggest that these enzymes are of significance in the diabetic state Bunting[97] found less than normal amounts of alkaline phosphatase in the kidney of alloxan-diabetic rats Dempsey [97a] has found alkaline phosphatase in the islets of Langerhans and has shown it to be present in greater concentration at the periphery of the islet in the rat

**Heredity** —This is the most important predisposing factor of all There is a statistically significant higher incidence of diabetes among blood relatives of diabetic patients than in the general population The evidence and implications are discussed by White and Pincus [98] They believe that the inheritance follows a Mendelian recessive pattern When both parents have diabetes all the children may be expected to develop the disease if they live long enough when one parent has diabetes and the other is a diabetic carrier 40 per cent of their children may develop the disease whereas if a diabetic or a carrier marries an individual who neither has diabetes nor is a diabetic carrier none of the children will have diabetes The implications of the last statement from the public health point of view are tremendous outbreeding of the disease by the consistent union of descendants of diabetic patients with those of nondiabetic families is theoretically possible

betes, in man and in the experimental animal However, all forms of *permanent* diabetes in experimental animals are characterized by lesions of the islets of Langerhans, and it seems probable that irreversible human diabetes is also fundamentally pancreatic, *i e*, essentially an insulin deficiency disease [illegible]

er[9] has recently made an able defense of the unitary pancreatic view of human diabetes The rôle of the H-G factor if any, in human and experimental diabetes is yet to be determined

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is difficult to conceive of long-continued and profound functional pathology without organic change, the field of mental disease excepted.

One may guess, of course, that these cases fall into the group in which even though *insulin secretion is normal the demand greatly exceeds the supply*, or in which the normally formed insulin may be inactivated (by insulinase?). It is more likely, perhaps, that newer histological methods, particularly silver impregnation, the phosphotungstic acid hematoxylin method of Hultquist and Tegner, and Gomori's new methods (*see Appendix*) *will show that many of these cases with apparently normal islets actually* have a marked reduction in the number of $\beta$ cells or an increase of $\alpha$ cells or both (*see* Chapter 3). Also the total volume of the islet organ[49,50] may be altered.

It is possible that some other mechanism may be operative, for instance a circulatory one. Since the amount of insulin demanded fluctuates between wide limits, some rapidly functioning regulatory mechanism must exist for the output of insulin. Obviously the amount utilized in absorbing a heavy meal would produce marked hypoglycemia if secreted in a fasting period; the intravenous glucose solution so frequently used by surgeons puts a heavy and sudden demand on the insulin producing mechanism, yet this is readily met. In the absence of prompt control we would swing ceaselessly and dizzily from hyperglycemia to hypoglycemia and back.

This regulation may be accomplished by alterations in rate of secretion which is partially dependent on the amount of blood passing through the islands, or by alterations in amount of blood flow which determines not only the nutrition of the island cells but the amount of insulin transported from the islands.

In examining the living pancreas of the mouse by a modification of Covell's[102] method, using transmitted light, one of us (S. W.) found in the thinner portions of the pancreas reddish white spots which faded slightly from time to time and others appeared. Subsequent histological examination indicated these spots to be islands of Langerhans. Thus we may have an intermittent circulation of blood through the islands and a very simple and rapid means of regulating the output of insulin. Such an intermittent circulation might be expected in view of the known intermittent circulation in the glomeruli of some animals, the peripheral capillaries, and elsewhere.

Berg,[103] using reflected light, has reported similar results, emphasizing the variation in the capillary pattern and the rapidity with which these changes occur.

It is theoretically possible, therefore, that a circulatory disturbance might be responsible for failure of release of insulin from histologically normal islets. However, no such mechanism has been demonstrated.

## SUMMARY

It is obvious that innumerable factors may come into play in producing a *temporary* diabetic state or in accentuating an already established dia-

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pains to avoid even minor trauma and to protect any injury received even to a blister, from infection So long as his diabetes is under control and his circulation adequate he has little more to fear than a normal person Infection always makes an existing diabetes worse and inadequately controlled diabetes is an ideal setting for rapid extension of an infectious process especially of the pyogenic type As has been pointed out organisms of low pathogenicity as *Staphylococcus albus* and *E coli* may be highly dangerous to the diabetic

Uncomplicated physical injuries as fractures and clean wounds heal as well in the diabetic patient as in others However suddenly changed habits of exercise and diet may complicate the management of the diabetes

Acidosis and coma need not be a factor if adequate dietary control and insulin are utilized as needed However any dietary indiscretion or inadequacy of insulin is dangerous Infection is of major importance in precipitating coma Impending coma impairs judgment and responsibility

Arteriosclerosis usually gives ample warning of its presence in the arteries of the legs Cleanliness and maintenance of warmth are essential to prevent gangrene When arteriosclerosis involves the coronaries however a fatal occlusion may be the first inkling of its presence Avoidance of sudden fluctuations of blood sugar and undue exertion are useful protective measures

Occupations requiring long and irregular hours without opportunity for control of the diet and use of insulin should be avoided by the diabetic patient

## INSULIN REACTIONS

Insulin reactions usually are not fatal A man aged seventy years whose left leg had been amputated at mid thigh for gangrene sat up in bed Feeling faint he called to others in the ward I m having an insulin reaction and fell dead His post mortem examination revealed an embolus in his pulmonary artery and a slightly elevated blood sugar Case 32 772 supposedly died of an insulin reaction but the autopsy showed a fresh coronary occlusion Case 36 597 died suddenly twelve days after a fracture of the femur An insulin reaction was suspected but the autopsy revealed the pulmonary artery occluded by an embolus Such cases could be readily multiplied No death should be ascribed to insulin without an autopsy and blood sugar determination

There are positive changes in death due to excess of insulin as have been described in Chapter 22

Aside from fatal insulin reactions hypoglycemic coma complete or partial, is of much significance The clinical picture may simulate drunkenness Hyperinsulinism may be precipitated by delay in obtaining food by sudden amelioration of an infectious process by mistake or carelessness in dosage, or by intent The hypoglycemic person may lose consciousness wholly or in part It is difficult to estimate at what point he ceases to be

## Chapter 27

# MEDICO LEGAL ASPECTS OF DIABETES MELLITUS

THE medico legal aspects of diabetes may be considered under four headings the etiology of the disease in relation to trauma and occupation special hazards of the diabetic state effects of insulin particularly hyper insulinism the diagnosis of the disease from post mortem material

While one s sympathy may well be aroused by the plight of a diabetic patient who may believe his disease resulted from trauma or some occupational hazard or was *aggravated thereby any such claim should be most* carefully scrutinized not only for the sake of scientific accuracy but also for the sake of the hundreds of thousands of diabetics who will be hampered in obtaining gainful employment should the risk and cost of compensation of certain of them prove too high

Rigid control of specimens of blood and urine used in establishing the presence or absence of diabetes must be maintained

### ETIOLOGY

Trauma may be claimed but is not a significant factor in light of our

betes following head injuries may be based Certainly temporary disturbances *in carbohydrate metabolism may follow injury of any* kind but this is to be regarded as a nonspecific reaction to stress As noted in the preceding chapter no increase in diabetes was found in either World War

Psychogenic origin for diabetes has not been proved in spite of the evidence that emotional factors cause exacerbation of the disease[3] *and might* theoretically act as the precipitating factor in a predisposed person

Trauma may lead to persistent infection as osteomyelitis[4] which might predispose to the development of diabetes

Occupation is not known to be a significant factor in the causation of diabetes

Extensive discussion of the medico legal aspects of trauma may be found in Joslin s book[2]

### SPECIAL HAZARDS

The chief of these are infection acidosis and coma and arteriosclerosis The *diabetic must take special precautions against infection* taking

pains to avoid even minor trauma and to protect any injury received even to a blister, from infection So long as his diabetes is under control and his circulation adequate, he has little more to fear than a normal person Infection always makes an existing diabetes worse and inadequately controlled diabetes is an ideal setting for rapid extension of an infectious process especially of the pyogenic type As has been pointed out organisms of low pathogenicity, as *Staphylococcus albus* and *F coli* may be highly dangerous to the diabetic

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responsible Careful *clinical* study, reinforced by blood sugar determinations will aid in establishing this

Numerous examples of the *medico-legal* importance of insulin reactions are given in the discussion in Joslin's book [2]

## THE DIAGNOSIS OF DIABETES MELLITUS FROM POST MORTEM MATERIAL

Post *mortem* diagnosis of diabetes while sometimes impossible may frequently be made *definitely* It must be remembered the adequately treated diabetic may show no evidence of the disease *Post mortem* urine sugar determinations may be helpful as may the presence or absence of acetone bodies Absence of sugar in the urine should not be relied upon if the urine is heavily contaminated with bacteria or if marked acidosis exists as renal tubular damage may have caused a renal shut-down

The post mortem blood sugar is of great value In the absence of gross contamination the diminution of blood sugar may be very slow Thus in Table 49 we see the results obtained from defibrinated heart's blood kept at room temperature for varying lengths of time before analysis Although there was a sudden marked initial drop in one case the subsequent decrease was slow in both cases

Table 49 Decrease in Blood Sugar of Defibrinated Blood at Room Temperature

| Time in hours | *Blood sugar in Mg per cent* Case No 3 065 | Case No 3[illegible] |
|---|---|---|
| | *Ante morte* | |
| 3 5 | 400 | |
| 4 5 | | 230 |
| | *Post mortem* | |
| 1 25 | | 300 |
| 2 0 | 46 | |
| 4 0 | | 260 |
| 4 5 | 44 | |
| 5 5 | | 220 |
| 6 5 | 44 | |
| 8 0 | 43 | |
| 10 0 | 40 | |
| 13 0 | 24 | |
| 19 0 | | 140 |
| 23 5 | 16 | |
| 26 0 | | 130 |
| 28 0 | 16 | |

In Table 50 a number of ante-mortem and post mortem blood sugars are compared Thus the blood sugar may remain abnormally high and an evidence of diabetes for some hours after death (The fact that some of the post mortem values are higher than the ante-mortem may perhaps be explained by some of the considerations mentioned below )

TABLE 50.—COMPARISON OF ANTE MORTEM AND POST MORTEM BLOOD SUGAR VALUES

| *Pathological No* | *Ante mortem blood sugar* | *No. of hours to death* | *No. of hours to autopsy* | *Post mortem blood sugar* |
|---|---|---|---|---|
| 15079 | 250 mg % | 3 | 1.25 | 190 mg % |
| 16771 | 1000 | 2.5 | 0.66 | 780 |
| 17720 | 920 | Few | 1 | 940 |
| 18359 | 210 | Few | 2.5 | 240 |
| 18453 | 940 | 4 | 2 | 210 |
| 19042 | 270 | 0.5 | 1 | 320 |
| 19342 | 990 | 3 | 0.5 | 1000 |
| 20175 | 130 | 5 | 1.5 | 30 |
| 21531 | 340 | 5.75 | 1.25 | 200 |
| 21662 | 320 | 4 | 1.5 | 220 |
| 22683 | 260 | Few | 1 | 80 |
| 23085 | 830 | 4 | 2 | 790 |
| 24638 | 340 | Few | 2.5 | 280 |
| 2515 | 100 | Few | 1.25 | 270 |
| 27095 | 190 | 1.5 | 3.5 | 320 |
| 28681 | 340 | Few | 2 | 320 |
| 28809 | 150 | 4 | 1.33 | 100 |
| 29764 | 690 | Few | 0.66 | 440 |
| 31954 | 290 | Few | 1 | 230 |
| 32990 | 520 | 12 | 5 | 270 |
| 33834 | 180 | Few | 1 | 160 |
| 11864 | 370 | 2 | 1 | 520 |

It is quite important that blood be drawn from the *left* side of the heart at autopsy. Blood in the right side may show high levels of reducing substances presumably due to proximity of the column of blood to the liver and diffusion of glucose from breakdown of glycogen in the latter organ. Hamilton Paterson and Johnson[5] state that values over 200 mg per 100 ml in the left heart may be taken to signify ante-mortem hyperglycemia. However Hill[6] and Fisher[7] caution that agonal release of epinephrine from the adrenal glands may lead to a misleadingly high terminal blood sugar even in the left heart.

Naumann[8] has emphasized the value of using cerebrospinal fluid (obtained by cisternal puncture). Presumably the rate of glycolysis is less than that of blood. Probably also agonal changes are less marked. Here a level of over 200 mg per cent is probably significant. The presence of acetone suggests coma as the cause of death.

Histological study may reveal changes in the pancreas but as was pointed out in Chapter 4 these may not be pathognomonic of the disease. Cell counts by the newer methods (see Appendix) may be of value. It seems reasonable to state that if the ratio of $\beta$ to $\alpha$ cells can be shown to be less than 3 a presumptive diagnosis of diabetes can be made. Toreson[9] holds that the demonstration of glycogen in the islands is positive morphological evidence of diabetes. Degranulation of the $\beta$ cells alone is apparently regarded by Bell[10] as a reliable diagnostic criterion. Conceivably a large terminal infusion of glucose might be misleading since Peterson[11] was able

to produce degranulation of $\beta$ cells in experimental animals in fifteen minutes by massive infusion of glucose.

In the inadequately treated diabetic, deficient liver glycogen together with glycogen in the liver cell nuclei and the renal epithelium may give the clue. Much glycogen may be present in the kidney in von Gierke's disease. That the carbohydrate content of liver tissue changes but little after the early post-mortem alteration is shown by the following table taken from Popper and Wozasek.[17]

TABLE 51.—TOTAL CARBOHYDRATE CONTENT OF LIVER IN RELATION TO TIME POST MORTEM[17]

| *Hours post mortem* | *Content* | *Twenty-four hours later at room temperature* | *Twenty-four hours later at icebox temperature* |
|---|---|---|---|
| 18 | 0.83 | 0.84 | 0.90 |
| 17 | 1.07 | 1.08 | 1.16 |
| 17 | 0.37 | 0.33 | 0.35 |

At this point it is well to emphasize that special alcoholic fixatives, while useful, are not necessary for the demonstration of glycogen[9, 13] (*see* Appendix). *Thus glycogen may be looked for in old paraffin blocks or in stored tissue.*

Other lesions which are, to say the least, strongly suggestive of diabetes and which may be conclusive if added to other findings are intercapillary glomerulosclerosis in the kidneys (Chapter 12) and capillary microaneurysms in the retina (Chapter 13).

As an example of the conclusiveness with which a post mortem diagnosis of diabetes can be made under favorable circumstances, the following case observed by one of us (P.I.) may be cited. *The patient was a woman of fifty who died of a coronary occlusion. She was comatose during the last few hours of life, having been taken moribund to a hospital. No suggestion of diabetes appeared in her history. At the time of autopsy the tissues had a sweetish odor (probably acetone). Urine taken from the bladder showed large amounts of sugar and acetone. Microscopic examination revealed the following: abundant glycogen in the renal tubules, occasional small granules of glycogen in the $\beta$ cells of the islands of Langerhans, a $\beta$ to a cell ratio of approximately 2.7 by the Gross-Schultze method, and typical intercapillary glomerulosclerosis in the kidneys.* There were thus no less than five significant findings, any one of which might be considered as adequate presumptive evidence of diabetes, while all five taken together establish the diagnosis beyond doubt, regardless of the negative clinical history. Incidentally, of course, a coronary occlusion in a woman of fifty is in itself sufficient to raise a very strong suspicion of diabetes (*cf.* Chapter 9).

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# Chapter 28

# A UNIFYING CONCEPT OF THE PATHOLOGY OF DIABETES MELLITUS

AT FIRST sight the protean and heterogeneous manifestations of the histopathology of diabetes and its complications as described in the preceding chapters seem utterly unrelated and confusing. The desirability of attempting to devise a unifying hypothesis is obvious. Friedenwald has made an interesting start toward such a concept in relation to the capillary lesions of the eye and kidney. It is the purpose of this chapter to suggest that his hypothesis may be extended to embrace practically all the important lesions characteristic of diabetes.

Friedenwald[1] in one of his brilliant papers describing capillary microaneurysms of the retina emphasized the extraordinary frequency with which these lesions occur in association with intercapillary glomerulosclerosis in the same individual. He pointed out (as did Ashton[2]) that the staining reactions of the hyaline deposits in both diseases were similar and cited the suggestion of McManus[3] that the hyaline of the renal lesions may represent a polysaccharide deposited from the circulating blood. He also noted that Jacobs[4] had described in the blood of diabetics a mucopolysaccharide the concentration of which closely paralleled that of blood glucose in response to insulin and cited the work of Pirani *et al.*[5] on amyloidosis in scorbutic guinea pigs as indicating a possible relation of capillary fragility to a disturbance of polysaccharide metabolism. These bits of evidence, although admittedly tenuous, suggested to Friedenwald that a more extended analysis of mucopolysaccharide metabolism was justified in relation to diabetes and diabetic retinopathy.

Reflection suggests that most of the characteristic tissue changes seen in diabetes may be interpreted in terms of disturbance of metabolism (or structural organization) of a simple polysaccharide (glycogen) or of the more complex carbohydrates known as mucopolysaccharides. In this connection one may consider changes in the pancreas, the larger arteries, the kidney, the eye and the skin.

The two most important lesions of the pancreas are hydropic change and hyalinization of the islets. If it be assumed that the work of Toreson[6] cited in Chapter 3 is generally applicable, then hydropic change must be regarded as due at least in part to the deposition of glycogen. If one places the amorphous substance in hyalinized islets in the category of amyloid (*see* Chapter 3) and if one accepts the view of Hass[7] of Jones

and Frazier,[8] and others that amyloid may consist at least in part of mucopolysaccharide, then one may perhaps consider hyalinization of the islets as due to the deposition of a polysaccharide-protein complex from the blood stream.

No adequate evaluation or even description of the vascular lesions of diabetes is possible without consideration of the properties of the little-understood "ground substance" and of basement membranes. Several excellent papers based on newer histochemical methods have appeared within the last year or so on this subject. Many of these are included in the New York Academy of Sciences symposium on "The Ground Substance of the Mesenchyme and Hyaluronidase."[9] Gersh and Catchpole[10] consider the ground substance of connective tissue and the homogeneous component of basement membrane to be closely related substances containing glycoproteins which form an optically homogeneous medium of fluid to gel-like consistency. These substances infiltrate and enclose a network of oriented fibrils. They are probably polymerized and presumably structurally organized on a submicroscopic level and, according to these authors, the degree of polymerization varies depending on age, activity and degree of pathologic change. The chemistry and distribution of these substances is reviewed by Meyer,[11-13] and some of the histochemical properties are discussed by Dempsey *et al.*,[14] Duran-Reynals *et al.*,[9] Leblond[15] and Ritter and Oleson.[16]

In various theories and discussions of arteriosclerosis of the larger arteries the ground substance has figured prominently. A swelling of the ground substance of the intima is an integral part of the imbibition theory of Virchow and Aschoff (*see* Chapter 9). Recent papers which have emphasized its importance include those of Faber,[17] of Meyer,[18] and of Rinehart and Greenberg.[19] It is entirely possible, if not probable, that the earliest lesion of atherosclerosis involves a subtle physicochemical change in the metachromatic gel or fluid which seems to encase the collagenous and elastic fibers of the arterial wall.

As regards the medial calcification of Monckeberg, it may be said that the earliest human lesion is unknown, although calcium salts often seem to be deposited first in elastic fibers. The medial calcification produced in animals by excessive doses of vitamin D has been studied in an effort to throw some light on the question, and it is interesting that at least two authors[20,21] describe the earliest visible change as a swelling of the interstitial ground substance.

Various points of view concerning intercapillary glomerulosclerosis or the Kimmelstiel-Wilson lesion of the kidneys have been discussed in Chapter 12. Regardless of whether one considers the amorphous hyaline material to be intercapillary or intracapillary, to be a deposit or a thickening of a pre-existing membrane, there can be no doubt that it is closely related to the substances under discussion. As noted in Chapter 12, McManus has shown that the characteristic staining qualities of this material are removed

by treatment with pectinase, and he suggests that the deposit may be derived from a polysaccharide circulating in the blood. In this connection it would seem desirable to pursue the studies of Jacobs[4] and others[22, 8] on the circulating mucopolysaccharides of the blood plasma. Jacobs,[4] studying the bound glucosamine of the blood in diabetic patients, concluded that the mucopolysaccharide yielding this glucosamine was somehow involved in the diabetic defect and that it responded to insulin in roughly the same manner as glucose itself as far as the direction, speed, and magnitude of the response were concerned.

The masterly studies of Friedenwald on the eye have been cited in Chapter 13. As noted above, it was he who suggested a close relationship between the capillary lesions in both the glomeruli and the retina. Certainly both lesions involve capillary basement membranes, and there appears to be considerable similarity between the hyaline nodules in the glomeruli and in the advanced microaneurysm of the retina. The presence of true aneurysmal dilatation of glomerular capillaries is not so clear, however,[2] although Allen[27] describes some degree of capillary dilatation as characteristic of the renal lesion.

The relation of basement membrane and "capillary cement substance" to capillary fragility is not clear. However, that such a relation is present has been suspected since the classical work of Wolbach[28] on the intercellular substance in experimental scurvy.

Finally, as regards the skin, certain random observations in this laboratory (cited in Chapter 9, page 146) have suggested a possible increase in basophilic ground substance in the corium in some cases of diabetes. As noted there, Unna has described what appears to be the same substance under the name of "collacin."

Recently Altshuler and Angevine[29] have published an interesting survey of the possible role of acid mucopolysaccharide in degenerative diseases of connective tissue with special reference to the "serous inflammation" of Rössle[30] and Eppinger.[3] They point out the probable importance of acid mucopolysaccharides in diseases involving connective tissue and characterized by such substances or processes as "hyalin," "amyloid," "sclerosis," and "fibrinoid degeneration." Obviously many of the changes occurring in the diabetic organism would fall within this group of diseases.

It seems likely that the newer physicochemical methods for studying the finer structure of connective tissue reviewed recently by Gross[3] may throw light on this important problem. An extension of the use of the electron microscope from normal to diseased connective tissue has recently been made by Gale.[33]

In summary, it appears that Friedenwald's concept may be extended to encompass all the major lesions characteristic of diabetes in the sense that all of them involve either simple polysaccharides (glycogen) or polysaccharide complexes (mucopolysaccharides), the latter probably often linked

to proteins The hypothesis is admittedly tenuous but may prove useful if supported by future investigations

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# APPENDIX A

## AUTOPSY AND STAINING METHODS

Through careful studies made post mortem on the bodies of diabetic patients the clinical information obtained during the course of their disease can be correlated with the anatomical findings and the knowledge thus gained made available for other sufferers. As Joslin says "An operation during life is attended by pain and is for the benefit of the individual; an operation after death is free from pain and is for the benefit of humanity."

Clinical observation and evidence at autopsy suggested the correlation of diabetes mellitus with diseased islands of Langerhans. Clinical observation, *examination of surgically removed pancreatic tissue and autopsy* proved that the islands produced insulin. The same methods may some day enable us to unravel the as yet but dimly understood etiology of the disease.

To quote from the motto on the wall of the post mortem room "Mortui vivos docent"—the dead teach the living. The responsibility for the success *of the autopsy rests directly upon the pathologist.* His is the duty of seeing that all possible information is gleaned. His is the duty of seeing that the autopsy is performed in a seemly manner and that the needs of the undertaker are sympathetically considered. If he neglects the first, it is futile for the internist to obtain permission for the autopsy. If he neglects the second, it is impossible for the internist to obtain permission. An operation performed after the patient's death deserves as dignified surroundings *as one before death.*

There are certain features which must be especially considered in autopsies on diabetic patients. The weight of the patient is important and this is easily obtained. A small chain hoist is hung from the ceiling and a spring scale (Chatillon) suspended from this with a spreader and four hooks below. *The truck on which the body lies is grasped by the hooks and the whole* raised by the chain hoist. A tare hand previously set for the weight of spreader, hooks and truck permits direct reading of the net weight.

Since the pancreas is extremely labile and since glycogen in tissues rapidly hydrolyzes, it is essential that the autopsy be performed as soon after death as practicable. If any long period of delay is anticipated, the body should be placed in the cold chamber promptly.

*The following autopsy technique is used in this laboratory.* A useful primary incision is the Y type, from over each acromial prominence to the sternum at the level of the third ribs, and thence in the mid line to the pubis, passing just to the left of the umbilicus. This affords an excellent

exposure and yet burial may be carried out in low necked [illegible] clothing [illegible] any trace of the incision visible

In an autopsy on a diabetic the pancreas should be [illegible] first [illegible] good exposure is obtained by cutting through the gastro[illegible] [illegible] The pancreas is freed at the tail and stripped upward and [illegible] avoiding all unnecessary trauma or compression The [illegible] most closely adherent to the duodenum is cut away [illegible] any abnormalities of the gland have been noted and [illegible] portion of the pancreas is removed and weighed [illegible] to 2 mm in thickness are cut and placed in the [illegible] sections of skin heart striated muscle and liver [illegible] priate fixative for subsequent staining for glycogen

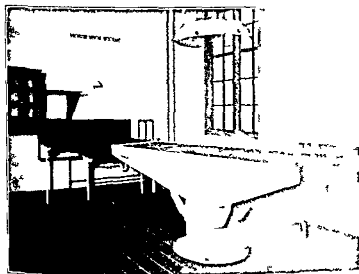


FIG 112 —Post mortem room for the De[illegible] [illegible] Memorial H[illegible] illustrating grill top-type table with [illegible] suction [illegible]

The autopsy may then proceed in the normal manner Farber[1] has given excellent accounts of the various procedures As soon as the heart is exposed a sample of blood is taken for blood sugar and nonprotein nitrogen determination Cerebrospinal fluid may also be taken by lumbar or cisternal puncture

Another variation from the usual routine is careful dissection of the remaining pancreatic tissue from the duodenum This is weighed its weight added to the other portion and sections are taken

A sample of bladder urine may be taken for glucose determination

A great assistance to the undertaker is the ligation separately of the in

nominate left common carotid and left subclavian arteries as well as the external iliacs The ends of the ligatures are left outside when the primary incision is sewed up

If permission has been given to open the head the carotid and vertebral arteries should also be ligated just within the cranial cavity

Routine fixation is most satisfactorily accomplished in Zenker s fluid and in 10 per cent neutralized formalin Absolute alcohol or better chilled Rossman s fluid * is used for all tissues in which the glycogen content is to be studied Chilled acetone may be used if enzyme studies are contemplated Helly s fixation is satisfactory for the hypophysis although hematoxylin and eosin after formalin give good results Mann s stain as modified by Dorothy Russell gives good differentiation of anterior lobe cells but has not in our hands given as consistent results as Biggart s pyrrol blue-eosin[3] (method below) Lillie s[4] eriocyanine-safranin or Crooke s[5] modification of Mallory s aniline blue stain

The following additional fixing fluids may be used for the pancreas

1 Bouin s fluid (especially useful for Gomori s stains)
2 Zenker s fluid without acetic acid
3 Helly s fluid
4 Regaud s fluid
5 Formol sublimate (formalin 1 part mercuric chloride 3 parts water 3 to 6 parts)
6 Carnoy s fluid
7 Champy s fluid

| | | |
|---|---|---|
| Potassium dichromate | 3 per cent | 21 cc |
| Chromic acid | 1 " " | 21 " |
| Osmic acid | 2 " " | 12 " |

8 Zenker osmic acid

| | | |
|---|---|---|
| Zenker s fluid without acetic acid | | 25 cc |
| Osmic acid | 2 per cent | 5 " |

9 Osmic chromic fluid

| | | |
|---|---|---|
| Osmic acid | 2 per cent | 4 cc |
| Chromic acid | 1 " " | 30 " |
| Acetic acid | glacial | 1 " |

All pancreatic tissue to be fixed in fluids containing osmic acid should be freed from fat as far as possible

Oil of cedar rather than chloroform is best for clearing the pancreas Dioxane gives good results

* Rossman s fluid to 9 parts of saturated picric acid in absolute alcohol add 1 part of concentrated formalin just before use Stock solution is kept in the icebox and specimen bottle containing the tissue is immediately returned to the icebox This chilling tends to prevent the piling up of glycogen on one side of the cell due to rapid diffusion of the fixing fluid

Of course the smaller the blocks of tissue the easier it is to get thin sections and on properly fixed material sections 2.5 micra thick can be fairly readily obtained. A large number of different stains may be utilized. We have found the following list satisfactory.

For general examination

1. Phloxine-methylene blue or eosin methylene blue[6]
2. Hematoxylin and eosin

For demonstration of fibrosis and hyalinization

1. Mallory's aniline blue[6]
2. Mallory's phosphotungstic acid hematoxylin[6]
3. Masson's trichrome[6] or Gomori's new trichrome[7]

For demonstration of specific granules

1. Gomori's chrome alum hematoxylin[8] (method below)
2. Gomori's aldehyde fuchsin[9] (method below)
3. Bensley's[10] modification of Mallory's aniline blue stain (method below)
4. [illegible]
5. [illegible] (method below)
6. [illegible] [met]hod below)
7. [illegible] phosphotungstic acid hematoxylin (method below)

*Note*. The first four of these methods require reasonably fresh tissue and work better after special fixatives (Bouin's, Helly's, or formol sublimate). The last three are applicable to old formalin fixed material and to routine autopsy material fixed some hours after death. In fact the Gros-Schultze method does not work well on tissue which is fixed less than six hours post mortem. We have obtained good results with it on tissue stored in formalin for several years.

For demonstration of mitochondria

1. Regaud's iron hematoxylin[6]
2. Altmann's aniline fuchsin methyl green[6,7]

For demonstration of glycogen and mucopolysaccharides

1. Best's carmine stain*[14]
2. Periodic acid and Schiff reagent as described by McManus[15] and by Hotchkiss*[16]

For the retina (microaneurysms)

1. Friedenwald's modification[7] of Hotchkiss' method (method below)

The diagnosis of hemochromatosis in a living patient is sometimes uncertain and its importance is so great from the standpoint of prognosis and from the probability of insulin resistance that biopsy is justifiable. Skin may be used but will often give a false negative result. Liver biopsy

* Stains for glycogen must be controlled by digestion of duplicate sections with buffered malt diastase solution. [?] (See also the Studies of A. M. Carpenter et al. Arch Path [?] 01 450 1951 and I. R. D. Lillie Bull. [illegible] Med Museums [?] 23 1947

is preferred. This should be fixed in buffered formalin (pH 7) or alcohol formalin embedded in paraffin sectioned stained for iron with potassium ferrocyanide (Prussian blue reaction),[4] and counterstained with basic fuchsin. Granules of hemofuscin or hemosiderin present may be readily demonstrated by this method. Gillman and Gillman[13] recommend a fresh mixture of equal parts of 2 per cent potassium ferrocyanide and 5 per cent hydrochloric acid in 70 per cent alcohol. The mixture is heated to boiling before use. Staining is carried out for one to two minutes followed by counterstaining with 1 6000 basic fuchsin.

## METHODS

### Gomori's Chrome Alum Hematoxylin[5, 19]

Fix thin slices in any aqueous fixative Bouin's fluid or formalin sublimate (formalin 1 part saturated mercuric chloride 3 parts water, 3 to 6 parts) are preferable. Embed in paraffin or celloidin. Run thin paraffin sections through xylene and alcohols to water. They should be protected by dipping the slide in a dilute (0 5 to 1 per cent) solution of collodion in alcohol-ether before the last alcohol. Stain celloidin sections floating (unattached). Remove if necessary mercury precipitates with Lugol's solution.

1 Treat sections with Bouin's solution containing 3 to 4 gm of chrome alum per 100 cc at 37° C for twelve to twenty four hours. Wash under the tap until the color of picric acid is completely gone.

2 Oxidize sections with the following mixture for one to two minutes 2 5 per cent solution of potassium permanganate and 5 per cent solution of sulfuric acid 1 part each distilled water 6 to 8 parts. Rinse.

3 Bleach with a 1 to 3 per cent solution of sodium bisulfite or oxalic acid. Wash under the tap.

4 Stain in following solution for about five to ten minutes or longer until β cells stand out dark blue against a much paler background.

Mix equal volumes of 1 per cent aqueous hematoxylin and 2 per cent chrome alum solutions. Add to each 100 cc of the mixture 2 cc of 5 per cent potassium bichromate and 1 cc of 5 per cent sulfuric acid. The mixture is ripe after forty-eight hours and will keep in the icebox for many months. It should have a deep opaque somewhat purplish blue shade. Filter before use. (Ripening can also be achieved by bringing the mixture to a short boil.)

5 Differentiate in 0 5 per cent hydrochloric acid alcohol for about one half minute. Wash under the tap for two to three minutes.

6 Counterstain with 0 5 per cent aqueous solution of phloxin for two or three minutes. Rinse.

7 Immerse in a 5 per cent solution of phosphotungstic acid for one to two minutes.

8 Wash under the tap for five minutes. The section should regain its red shade.

9 Differentiate in 85 to 90 per cent alcohol until α cells stand out deep red.

10 Transfer to 95 per cent and absolute alcohols clear in xylene and mount in balsam.

Result β cells deep blue α cells bright red (*See* Plate III.) The D cells are not distinguishable being red like α cells. Also the α cells do not always stain well in human pancreas. Dr Sergio Bencosme[20] has obtained very beautiful results in animal material with a modification which consists essentially in staining with ponceau fuchsin after step 5 rinsing in 1 per cent acetic acid differentiating in

1 per cent phosphomolybdic acid placing again in 1 per cent acetic acid for one minute then dehydrating in 3 changes of absolute alcohol He uses a modified Zenker formol for fixation

### Gomori s Aldehyde Fuchsin*

This stain under proper conditions gives brilliant results imparting a deep purple shade to the granules of the $\beta$ cells (*See* Plate III ) Elastic tissue and mast cells are also stained Almost any fixative may be used but formalin formol-sublimate and Bouin's fluid are preferred

3 Rinse in water followed by alcohol 70 per cent

4 Stain fifteen to thirty minutes or longer even overnight in a Coplin jar filled with aldehyde fuchsin

The dye is prepared as follows add 1 cc concentrated hydrochloric acid and 1 cc of paraldehyde U S P to 100 cc of a 0 5 per cent solution of basic fuchsin in 60 to 70 per cent alcohol The color changes gradually (in about twenty-four hours at room temperature) to a deep purple almost indistinguishable from that of gentian violet and is then ready for use Gomori states that solutions keep for about four weeks at room temperature and for over six months in the icebox Fresh solutions stain faster than old ones The stain may be rinsed off with alcohol and the slides inspected during staining

5 Rinse in several changes of alcohol (concentration not stated) and counter stain as desired A trichrome method such as Gomori s or Masson s[4] containing a green not a blue dye is satisfactory Here again difficulty may be encountered in staining the $\alpha$ cells The D cells are said to take the green of the counter stain

### Bensley's Modification[8] of Mallory s Aniline Blue Stain

1 Altmann [illegible]

5 Dehydrate in alcohols, clear and mount

### Terbrüggen's Modification[1] of Bensley s Acid Fuchsin—Methyl Green

1 Either (a) fix in Helly s fluid or (b) soak thin pieces of formalin fixed tissue in 3 per cent potassium bichromate for a week

2 Stain paraffin sections two hours in a 10 per cent dilution of a saturated (about 12 per cent) solution of acid fuchsin

3 Differentiate for one-half to one minute in 2 per cent aqueous methyl green

4 Dehydrate rapidly, clear and mount

We have had occasional good results with this method The $\alpha$ cells are red $\beta$ cells bluish violet

### Gros Schultze Silver Impregnation

This important method originally intended for nerve fibers and popularized by Ferner,[11] has been subjected to extensive study by Hultquist and co-workers[12] Their modification is given here

Blocks of pancreas not more than 4 to 5 mm thick are fixed 6 hours or more post mortem in 10 per cent formalin Tissue from experimental animals (freshly killed) must be rolled in a piece of skin to protect from drying and kept at room temperature for several hours

1 Make frozen sections receive in distilled water, wash twice in fresh distilled water Sections must remain at least fifteen minutes in distilled water

2 Very fatty pieces of pancreas are treated twice for fifteen seconds in pure *pyridine Then wash in fresh distilled water until water is entirely free from odor*

3 Sections are transferred with a glass rod to 20 per cent silver nitrate The

material It is about one to five hours for rats fetuses and newborns about three to twelve hours for adults

4 Sections are put in aqueous formalin diluted 1 to 4 in a series of dishes and changed until no more white clouds come off They shoud not be in formalin over 10 minutes

5 Sections are transferred to ammoniacal silver nitrate (*see below*) made as in the original method except that not a single drop of ammonia beyond that neces

t (sa)
drop
ot a ld
ortant

fusely black

6 *Sections are transferred to dilute ammonia* (2 parts ammonia and 8 parts
nd mounted
nitric acid
n chloride
ing in gold
arried out
to be in
herent in all silver methods however, with a little practice, la reproducible results can be obtained

**Hultquist and Tegner s Modification[14] of Mallory s Phosphotungstic Acid Hematoxylin**

1 Cut frozen sections of formalin fixed tissue and receive in distilled water

2 Place sections in 0 5 per cent potassium permanganate for one to one and one-half minutes

3 Bleach in 0 25 to 0 5 per cent sodium bisulfite (one-half to four minutes)
4 Wash quickly in 3 changes of distilled water
5 Treat for three to four hours in a 2 to 5 per cent sol ition of ferric ammoni im sulfate

clouds disappear

the same as the method used by Gomori[8] for paraffin sections of Bouin fixed pancreas

### Friedenwald's[17] Method for Whole Mounts of Retina

The retina may be dissected out from the eye after fixation in formalin or other fixative and may be recovered from tissue already embedded in paraffin by melting the block in the oven and carrying the tissue back through xylol and alcohols to water In either case the delicate translucent retina should be carefully separated from the deeply pigmented choroid It is washed in tap water for twenty four hours then in distilled water

1 Place the retina (usually conveniently cut into about four pieces) in Hotchkiss

solve

uva

3 Place in reducing rinse for twenty minutes (Dissolve 1 gm potassium iodide and 1 gm sodium thiosulfate pentahydrate in 20 cc distilled water Add with stirring 30 cc ethyl alcohol then 0 5 cc 2 N hydrochloric acid A precipitate of sulfur slowly forms and is allowed to settle out but the solution may be used immediately)

4 Wash in distilled water thirty to sixty seconds

more 2 N hydrochloric

mixture spontaneously

Preserve in the dark

in two changes of absolute alcohol for ten minutes each clear in xylol and mount in balsam nerve-fiber layer up

The capillary walls and microaneurysms are brought out with remarkable clarity (See Fig 87 and Plate III)

### Biggart's Pyrrol Blue Eosin Method for Pituitary[24]

1 Fix in Zenker formol eighteen to twenty four hours
2 Stain thirty to thirty five minutes at 50° C in a mixture of equal parts of 0 5 per cent aqueous eosin Y and 0 3 per cent pyrrol blue (isamine blue)
3 Differentiate in the following solution 5 per cent sodium carbonate 10 parts absolute alcohol 40 parts (Differentiation is often quite rapid and must be watched carefully )
4 Dehydrate quickly clear and mount in a neutral medium

Result Acidophil cells red basophils deep blue chromophobes light blue

## OTHER HISTOCHEMICAL METHODS

Most of the important new methods for enzymes and the like are cited in Pearse's review[22] and are described in detail by Gomori[23] Other references on the mucopolysaccharides are given in Chapter 28

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APPENDIX I. THE CAUSES OF DEATH OF [illegible] DIABETICS

| | Naunyn Era 18[illegible] to May 31, 1914 | | Allen Era June 1, 1914 to August 6, 1922 | | Banting Era August 7, 1922 to December 31, 1936 | | Hagedorn Era January 1, 1937 to December 31, 1943 | | Charles H. Best Era January 1, 1944 to April 27, 1949 | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Deaths | % of all cases | Deaths | % of all cases | Deaths | % of all cases | Deaths | % of all cases | Deaths | % of all cases |
| All Causes | 326 | 100.0 | 836 | 100.0 | 4061 | 100.0 | 3151 | 100.0 | 2299 | 100.0 |
| A. Coma present | 208 | 63.8 | 347 | 41.5 | 340 | 8.4 | 98 | 3.1 | 43 | 1.9 |
| B. Coma absent | | | | | | | | | | |
| 1. Cardio-renal vascular | 57 | 17.5 | 206 | 24.6 | 2200 | 54.2 | 2047 | 65.0 | 1612 | 70.1 |
| Arteriosclerotic | 57 | 17.5 | 203 | 24.3 | 2184 | 53.8 | 2035 | 64.6 | 1596 | 69.4 |
| a. Cardiac | 20 | 6.1 | 83 | 9.9 | 1206 | 29.7 | 1287 | 40.8 | 1054 | 45.8 |
| b. Nephritic | 11 | 3.4 | 32 | 3.8 | 190 | 4.7 | 140 | 4.4 | 161 | 7.0 |
| c. Apoplexy | 9 | 2.8 | 41 | 4.9 | 381 | 9.4 | 368 | 11.7 | 269 | 11.7 |
| d. Gangrene | 12 | 3.7 | 35 | 4.2 | 323 | 8.0 | 160 | 5.1 | 71 | 3.1 |
| e. Site unassigned | 5 | 1.5 | 12 | 1.4 | 84 | 2.1 | 80 | 2.5 | 41 | 1.8 |
| Other circulatory and rheumatic heart disease | 0 | 0.0 | 3 | 0.4 | 16 | 0.4 | 12 | 0.4 | 16 | 0.7 |
| 2. Infections, total | 24 | 7.4 | 106 | 12.7 | 557 | 13.7 | 345 | 10.9 | 153 | 6.7 |
| Pneumonia & respiratory | 14 | 4.3 | 64 | 7.7 | 281 | 6.9 | 187 | 5.9 | 93 | 4.1 |
| Throat and ear | 0 | 0.0 | 6 | 0.7 | 21 | 0.5 | 3 | 0.1 | 0 | 0.0 |
| Gall bladder | 0 | 0.0 | 4 | 0.5 | 20 | 0.5 | 17 | 0.5 | 5 | 0.2 |
| Appendicitis | 2 | 0.6 | 3 | 0.4 | 25 | 0.6 | 18 | 0.6 | 2 | 0.1 |
| Carbuncle | 6 | 1.8 | 13 | 1.6 | 40 | 1.0 | 16 | 0.5 | 0 | 0.0 |
| Kidneys, acute | 0 | 0.0 | 1 | 0.1 | 36 | 0.9 | 29 | 0.9 | 18 | 0.8 |
| Abscesses | 0 | 0.0 | 7 | 0.8 | 59 | 1.4 | 28 | 0.9 | 8 | 0.4 |
| Other infections | 2 | 0.6 | 8 | 0.9 | 75 | 1.9 | 47 | 1.5 | 27 | 1.1 |
| 3. Cancer | 5 | 1.5 | 32 | 3.8 | 353 | 8.7 | [illegible] | 8.8 | 205 | 8.9 |
| 4. Tuberculosis | 16 | 4.9 | 41 | 4.9 | 169 | 4.2 | [illegible] | [illegible] | 48 | 2.1 |
| 5. Diabetes | 8 | [illegible] | 56 | 6.7 | 120 | 3.0 | 99 | 3.1 | 61 | 2.7 |
| 6. Accidents | 0 | 0.0 | 7 | 0.8 | 84 | 2.1 | [illegible] | 1.8 | 41 | 1.9 |
| 7. Inanition | 1 | 0.3 | 18 | | 6 | 0.1 | 0 | 0.0 | 0 | 0.0 |
| *8. Suicides | 1 | 0.3 | [illegible] | [illegible] | 27 | 0.7 | [illegible] | [illegible] | 11 | [illegible] |
| 9. Insulin reactions | [illegible] | 0.0 | | 0.0 | 5 | 0.2 | 10 | 0.4 | 7 | 0.3 |
| 10. Other diseases | 6 | 1.8 | 41 | [illegible] | [illegible] | 4.9 | 119 | 3.8 | 113 | 4.9 |

* Deaths reported through April 27, 1949. Prepared by the Statistical Bureau of the Metropolitan Life Insurance Company.

# APPENDIX C

Causes of Death in 818 Diabetic Autopsies*

| | to 1924 | 1924 to 1930 | 1930 through 1937 | 1937 through 1948 | Totals Number | Totals Per cent |
|---|---|---|---|---|---|---|
| Total cases | 105 | 157 | 224 | 332 | 818 | 100 0 |
| A Coma present | 66 | 32 | 29 | 9 | 136 | 16 6 |
| B Coma absent | 39 | 125 | 195 | 323 | 682 | 83 4 |
| 1 Cardiorenal vascular lesions | | | | | | |
| a Arteriosclerotic | | | | | 268 | 32 8 |
| 1 Aneurysm | 0 | 0 | 1 | 1 | | |
| 2 Cardiac | 7 | 27 | 49 | 117 | | |
| 3 Cerebral | 2 | 5 | 12 | 10 | | |
| 4 Gangrene | 4 | 6 | 1 | 0 | | |
| 5 Mesenteric | 0 | 0 | 6 | 2 | | |
| 6 Renal disease, vascular | 0 | 3 | 2 | 13 | | |
| b Renal disease nonvascular | | | | | 5 | 0 6 |
| 1 Glomerular | 0 | 1 | 2 | 1 | | |
| 2 Tubular | 0 | 1 | 0 | 0 | | |
| 2 Infections | | | | | 213 | 26 0 |
| a Gas gangrene | 1 | 1 | 0 | 0 | | |
| b Meningitis | 0 | 2 | 2 | 2 | | |
| c Multiple abscess | 3 | 7 | 2 | 3 | | |
| d Pericarditis | 0 | 2 | 4 | 0 | | |
| e Peritonitis | 1 | 6 | 5 | 9 | | |
| f Pneumonia | | | | | | |
| 1 Bronchial | 4 | 12 | 9 | 13 | | |
| 2 Lobar | 2 | 4 | 2 | 2 | | |
| g Pyelonephritis | 0 | 1 | 1 | 11 | | |
| h Rheumatic fever | 0 | 0 | 1 | 1 | | |
| i Septicemia | 3 | 12 | 30 | 35 | | |
| j Syphilis | 0 | 1 | 0 | 0 | | |
| k Tuberculosis | 6 | 5 | 4 | 2 | | |
| l Empyema | 0 | 1 | 0 | 1 | | |
| 3 Cancer | 0 | 13 | 26 | 42 | 81 | 9 9 |
| 4 Hemochromatosis | 1 | 1 | 2 | 3 | 7 | 0 9 |
| 5 Pulmonary embolus | 0 | 4 | 12 | 17 | 33 | 4 0 |
| 6 Insulin reaction (hypoglycemia) | 0 | 0 | 3 | 1 | 4 | 0 5 |
| 7 Accident | 0 | 1 | 1 | 0 | 2 | 0 2 |
| 8 Other causes | 5 | 9 | 18 | 17 | 49 | 6 0 |
| 9 Undetermined | | | | 20 | 20 | 2 4 |

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